Hyperpolarized Pyruvate (^{13}C) Injection

INVESTIGATOR'S BROCHURE

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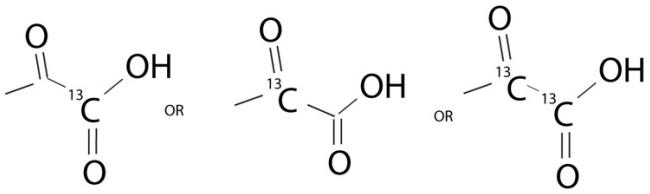
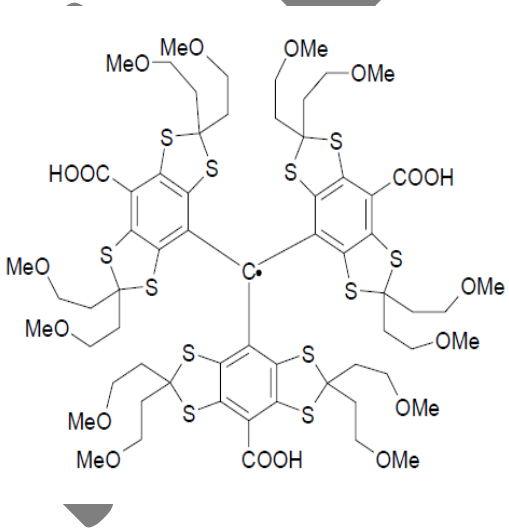
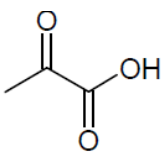
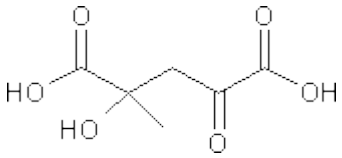
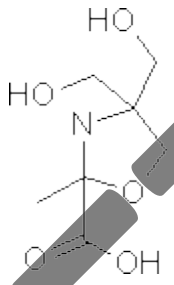
1. LIST OF ABBREVIATIONS

Term	Meaning
3D EPSI	Three-dimensional echo planar spectroscopic imaging
AE	Adverse event
AH110896	Pyruvic acid (2-oxopropanoic acid) (enriched with ^{13}C either the C-1 position, C-2 position, or both C1 and C2 positions, to NLT 99 %).  Initially, one AH number covered both [1-^{13}C]pyruvic acid and pyruvic acid in its natural abundance. The terms ^{13}C- AH110896 and ^{12}C-AH110896 were then used.
AH111501	Methyl, tris[8-carboxyl-2,2,6,6-tetrakis(2-methoxyethyl) benzo[1,2-d:4,5-d']bis[1,3]dithiol-4-yl]-, trisodium salt 
AH111501 sodium salt	Trisodium salt of AH111501, enables hyperpolarization of [1-13C]pyruvic acid
AH111710	**Pyruvic Acid** 
AH111710 sodium salt	**Sodium pyruvate**

Term	Meaning
AH112623	**Parapyruvate, 2-hydroxy-2-methyl-4-oxo-pentanedioic acid** 
AH112615	4,4-Bis-hydroxymethyl-2-methyl-oxazolidine-2-carboxylic acid 
AUC	Area under the curve
BPH	Benign prostatic hyperplasia
Bw	Body weight
CNS	Central nervous system
Cmax	Maximum concentration
CVS	Cardiovascular system
CRO	Contract research organization
CT	Computed tomography
Dissolution medium	TRIS/EDTA dissolution medium
DNP	Dynamic nuclear polarization
Drug product	Hyperpolarized Pyruvate (13C) Injection (clinical study
Drug product kit	For clinical studies GE-101-001/GE-101-003, kit containing
Drug substance	Active pharmaceutical ingredient, here [1-13C]pyruvic acid
ECG	Electrocardiogram
EDTA	Ethylenediaminetetraacetic acid
EPA	Electron paramagnetic agent, see AH111501 sodium salt
FastCSI	Fast chemical shift imaging
FIDCSI	Free induction decay chemical shift imaging
GLP	Good laboratory practice
GMP	Good manufacturing practice
HED	Human equivalent dose; the doses (in mg/kg) used in preclinical studies
hERG	Human Ether-a-go-go Related Gene; defined by ICH as the gene
HPLC	High-performance liquid chromatography
ICH	International Conference on Harmonization
id	Injected dose
im	Intramuscular
ip	Intraperitoneal
Iv	Intravenous
K	(degrees) Kelvin
LAF	Laminar air flow
LC-MS	Liquid chromatography mass spectrometry
Mixture of pyruvic acid and	Drug product kit component in clinical studies GE-101- 001/GE-101-
Mixture of [1-13C]pyruvic acid	Drug product kit component in clinical study GE-101-002

Term	Meaning
M	Molar
MLA	Mouse Lymphoma Assay
mM	Millimolar
µM	Micromolar
MR	Magnetic resonance
MRI	Magnetic resonance imaging
MS	Mass spectrometry
Na2EDTA	Disodium ethylenediaminetetraacetate
NaOH	Sodium hydroxide
Naturally abundant pyruvic acid	Pyruvic acid, see AH111710
Naturally abundant	Sodium pyruvate, see AH111710 sodium salt
NLT	Not less than
NMR	Nuclear magnetic resonance
NMT	Not more than
Nonclinical test item	Dissolved mixture of pyruvic acid and AH111501 sodium salt in
Osmotic control solution	Glucose or mannitol with osmolality matched to nonclinical test item
Parapyruvate	See AH112623
Ph.Eur.	European Pharmacopeia
PR-interval	The ECG interval (in milliseconds) from the beginning of the P wave to
Preclinical test item	See nonclinical test item
PSA	Prostate-specific antigen
pv	Perivascular
Pyruvate Injection	dissolved mixture of pyruvic acid and AH111501 sodium salt in TRIS/EDTA dissolution medium; solution for injection in clinical studies GE-101-001/GE-101-003
[1-^{13}C]Pyruvic acid	AH110896
Pyruvic acid	AH111710
^{13}C Pyruvic acid	[1-^{13}C]Pyruvic acid
^{12}C Pyruvic acid	Pyruvic acid
PC-3	Slow-growing prostate adenocarcinoma of human origin
QT-interval	The ECG interval (in milliseconds) from the beginning of the Q wave to the end of the T wave
QTcV	The QT interval corrected for heart rate according to Van de Waters formula
QWBA	Quantitative whole-body autoradiography
SNR	Signal-to-noise ratio
S9	A combination of cytosolic and endoplasmic reticulum fractions that possesses a number of drug metabolizing enzymes
T	Tesla
T_1	Time constant for hyperpolarization decay
T_{max}	Time of maximum concentration
TK	Toxicokinetics
tk locus	Thymidine kinase locus
TRAMP	Transgenic adenocarcinoma of mouse prostate
TRIS	Tris (hydroxymethyl) aminomethane, or trometamol
TRIS/EDTA vehicle control solution	Control solution used in some of the nonclinical studies.

Term	Meaning
TRIS/EDTA dissolution medium	Component of the drug product kit for nonclinical studies and clinical studies GE-101-001/GE-101-003: solution used to dissolve the non-hyperpolarized mixture of pyruvic acid and AH111501 sodium salt
TRIS/EDTA buffer solution	Component of the drug product kit for clinical study GE- 101-002; solution used to neutralize, buffer and dilute dissolved [1-[^{13}C]pyruvic acid during compounding of Hyperpolarized Pyruvate (^{13}C) Injection
UCSF	University of California in San Francisco
USP	United States Pharmacopoeia
Vehicle control solution	**TRIS/EDTA vehicle control solution**

Bold: preferred term

2. SUMMARY

Hyperpolarized Pyruvate (^{13}C) Injection, containing spin-polarized ("hyperpolarized") [^{13}C]pyruvate, is being studied as a diagnostic agent in combination with ^{13}C spectroscopic MR imaging. The aim is to visualize [^{13}C]pyruvate and its metabolites and thereby distinguish between anatomical areas with normal vs. abnormal metabolism, which should be useful in diagnosing and characterizing, for example, malignancy. Hyperpolarized Pyruvate (^{13}C) Injection and [^{13}C]pyruvate are general terms used throughout this brochure, that refer to all ^{13}C labeling patterns, such as [1-^{13}C]pyruvate, [2-^{13}C]pyruvate and [1,2-^{13}C]pyruvate. From biological and safety standpoints, pyruvate with each of the labeling patterns behaves identically in the human body [Koletzko et al., 1997].

A nonclinical program has been performed in which the safety of both pyruvate and AH111501 (Electron Paramagnetic Agent; EPA), the 2 novel drug product components of Pyruvate Injection, has been demonstrated. The program included expanded acute-dose studies in Sprague-Dawley rats, from which the No-Observed-Adverse-Effect-Levels (NOAELs) were estimated as 892 mg/kg bw for pyruvate and 17 mg/kg bw for AH111501. In an expanded acute-dose study in beagle dogs, the NOAEL for pyruvate was 446 mg/kg bw and 8.5 mg/kg bw for AH111501. The cardiovascular effects of formulations of pyruvate were studied in both conscious and pentobarbital/fentanyl-anaesthetized dogs. The conscious dog was considered the most relevant model for extrapolating results to healthy human volunteers and the anaesthetized dog as a sensitive model for extrapolating results to subjects with a compromised baroreflex function (as may be the case in elderly subjects with prostate cancer, which is one of the target populations in which use of Pyruvate Injection is planned). In addition, effects of pyruvate and AH111501 have been studied in local tolerance studies, genotoxicity studies and central nervous system studies. Based on the cardiovascular effects (vasodilatation leading to small, short-lasting but significant reductions in blood pressure and compensatory increases in heart rate) observed in the most sensitive model, the NOAEL for Pyruvate Injection is 1.4 ml/kg bw (or 100 ml for a 70-kg subject) of a 250 mM formulation (equal to 31 mg/kg bw of pyruvate). AH111501 was qualified in a separate toxicology program and the lowest NOAEL was 8.5 mg/kg bw (or 595 mg for a 70-kg subject).

The available nonclinical data indicated sufficiently good safety margins to support testing the safety and tolerability of a 250 mM formulation of Pyruvate Injection containing 3 µM AH111501 (EPA) (i.e., 22.0 mg/ml of pyruvate and 4.6 µg/ml of EPA) in healthy human male and female volunteers in a placebo-controlled phase 1 dose-escalating study up to doses of 0.71 ml/kg bw (i.e., up to 15.7 mg/kg bw of pyruvate and 3.3 µg/kg bw of EPA). The data from the nonclinical studies in conscious and anaesthetized dogs indicated that special attention should be paid to the possibility of mild cardiovascular effects occurring, like those reported when Pyruvate Injection was administered rapidly at large volumes to conscious dogs (increases in heart rate) and anaesthetized dogs (vasodilatation leading to small, short-lasting but statistically significant reductions in blood pressure and compensatory increases in heart rate).

GE Healthcare, the initial sponsor of this IND, has performed 2 Phase 1 clinical trials with Pyruvate Injection (GE-101-001 and GE-101-003), in young and elderly healthy volunteers respectively, to assess the safety and tolerability of Pyruvate Injection in humans. As no imaging was performed in these studies, Pyruvate Injection made from pyruvate instead of [1-^{13}C]pyruvate was used. These studies showed that doses of Pyruvate Injection up to 0.43 ml/kg (i.e., up to 9.4 mg/kg bw of pyruvate and 2.0 µg/kg bw of EPA) were equally well tolerated by young and elderly healthy male and female volunteers. However, in study GE-101-001, it was planned that doses up to 0.71 ml/kg of Pyruvate Injection would be assessed. The study was temporarily stopped by the sponsor after completion of dosing for the 0.57 ml/kg dose group due to the occurrence of non-serious AEs in 2 subjects ('unresponsiveness' in 1 case

and 'flushing' accompanied by changes in blood pressure and heart rate in the other case) that the principal investigator considered to be concerning and related to the administration of Pyruvate Injection. These events occurred in 2 of the 4 subjects in that dose group who received Pyruvate Injection. The sponsor decided not to proceed with the planned dose escalation to 0.71 ml/kg.

Based on the safety profile of Pyruvate Injection established in the 2 Phase 1 studies referred to above, it was justified to proceed with the next study: a phase 1/2a ascending-dose safety and tolerability and exploratory imaging study utilizing Hyperpolarized Pyruvate (^{13}C) Injection in patients with established prostate cancer. This study was not performed under the GE IND 76, 651 (now the NCI IND) but under an IND filed by UCSF.

UCSF, collaborating with GE, has completed a phase 1 study on hyperpolarized [1-13C]pyruvate injection in 31 subjects with prostate cancer (NCT01229618). This was an ascending-dose study to assess the safety and tolerability and imaging potential of hyperpolarized [1-13C] pyruvate Injection via 13C imaging (13C MRI) and 13C MR spectroscopic imaging (13C MRSI). Three dose levels of hyperpolarized [1-13C]pyruvate (0.14 ml/kg, 0.28 ml/kg and 0.43 ml/kg) with a minimum of 6 patients at each dose level were administered via single IV injection followed by MR imaging scans. The first 3 subjects underwent dynamic 13C MRI to define the kinetics of delivery and metabolism of hyperpolarized [1-13C]pyruvate. The second 3 subjects underwent 13C MRSI to obtain 3-dimensional (3-D) spatial information about metabolism of hyperpolarized [1-13C]pyruvate in regions of the prostate with and without cancer involvement.

Most groups in North America, Asia and Europe that are performing clinical trials with Hyperpolarized C-13 Pyruvate formulations are collaborating with each other through the UCSF's Hyperpolarized MRI Technology Resource Center (HMTRC) funded by the National Institute of Biomedical Imaging and Bioengineering. The groups meet regularly on-line and at a yearly workshop and share presentations, publications and data through the HMTRC website (https://radiology.ucsf.edu/research/labs/hyperpolarized-mri-tech).

These groups were polled in first quarter 2024 with a request to provide the number of patients and doses they had studied to the end of 2023 and to note if any serious adverse events had been observed. The summary is shown in Table 1. There have been 1,112 subjects studied as of 31 DEC 2023, and the total number of studies, including repeat studies) for both patients and volunteers reached 1,656. On average, three out of 10 studies involved volunteers.

Many of these studies represented multiple injections, as can be seen from Table 1, which has been expanded to capture 2, 3 and 4 or more injections in the same subject, with a significant percentage (greater than 40%) of repeated studies allowing test/retest and follow up of responses to therapy. This continues to attest to the high safety profile of the formulation, composed of 250 mM pyruvate and no more than 3 microM EPA delivered at a rate of 0.43 mL/kg body weight. All sites reported that no serious adverse events were observed. Note that these studies have not been performed under IND 76, 651. The reports are anecdotal, and they have not been verified. Only self-reported mild side effects associated with the injection and no moderate or severe adverse events even after multiple administrations to the same subject were reported. These appear much milder than those preliminarily reported in the assessed safety and tolerability study of Pyruvate injection in healthy male and female volunteers (protocol GE-101-001, Phase 1 placebo controlled randomized ascending dose study), and which served as the basis for the dose selection.

The applications have remained focused on cancer patients, with prostate, brain and other solid tumors, but cardiology with about 22% of all studies has gained high interest, both in evaluating the critical issue of chemotherapeutic cardiac toxicity as well as in diabetes research.

Sites continue to migrate to an improved Part B with the appropriate qualification runs and smoothly transitioned in the clinic, allowing the combination of multiple probes imaging, such as C-13 Pyruvate in the C-1 and C-2 positions. A major milestone was accomplished by the UCSF team in their co-injecting of hyperpolarized C-13 Pyruvate and N-15 urea in prostate cancer patients: https://clinicaltrials.gov/study/NCT06391034, ushering a new era of development. More probes are now in development, such as (13C) alpha-ketoglutarate (aKG) for the imaging of IDH mutant in glioma: https://clinicaltrials.gov/study/NCT05851378, 13C bicarbonate to measure tissue pH in prostate cancer: https://clinicaltrials.gov/study/NCT05851365, and 13C fumarate for cell death imaging, with their quality control facilitated by an MPQC (multi-probe quality control) unit that showed flexibility at an astonishing speed with the partial release of a drug product in a matter of seconds. Published clinical studies are summarized in section 6.4 Literature reported studies.

Table 1: Summary of All Clinical C-13 Pyruvate Hyperpolarization Studies Performed Since Program Initiation:

ALL RECEIVED DATA				
C-13 Pyruvate Hyperpolarization	**As of 31 December 2023**			
	Patients with one or more injections	Patients with two or more injections	Patients with three or more injections	Patients with four or more injections*
Volunteers	305	151	37	27
Patients	807	254	49	15
TOTAL	**1112**	**405**	**86**	**42**
The Patients breakdown is as follows:				
Brain tumors (glioma, other)	192	68	7	1
Prostate related tumors	281	29	4	1
Other solid tumors (pancreas, ovarian, cervical, thyroid, breast,…)	170	62	6	1
Traumatic Brain injury	10	9	2	2
Cardiac related (diabetes, cardiotoxicity, cardiac function)	135	78	29	10
Other	19	8	1	0
TOTAL	**807**	**254**	**49**	**15**

ALL RECEIVED DATA				
C-13 Pyruvate Hyperpolarization	**As of 31 December 2022**			
	Patients/Volunteers with one or more injections	Patients/Volunteers with two or more injections	Patients/Volunteers with three or more injections	Patients/Volunteers with Four or more injections
Aarhus	39	10	0	0
Cambridge	117	37	1	1
CGMH, Taipei	11	4	0	0
Maryland	2	0	0	0
MD Anderson	6	3	0	0
MSKCC	35	11	0	0
Nottingham	24	1	0	0
Oxford	59	23	1	0
Singapore	0	0	0	0
Stanford	4	2	0	0
Sunnybrook	85	24	0	0
UC London	91	3	0	0
UCSF	470	162	36	12
UT Southwestern*	155	118	48	29
Zurich	14	7	0	0
TOTAL	**1112**	**405**	**86**	**42**

** An additional 21 studies have been completed at UT Southwestern with volunteers receiving five, six, seven, eight and nine injections as follows:*

5 injections	6 injections	7 injections	8 injections	9 injections
5	3	1	1	1

As of the time of this report, there are 52 trials posted on https://clinicaltrials.gov/ Clinicaltrials.gov (Table 2), 8 trails posted on clinicaltrialsregister.eu clinicaltrialsregister.eu (Table 3) and 1 trial posted on WHO or ORCID (Table 4). None of these trials are sponsored under IND 76,651, but are sponsored by the different institutions.

Table 2. Trials listed on Clinical Trials.gov

Title	Status	Disease	Institution	Link
Molecular Imaging and Spectroscopy with Stable Isotopes in Oncology and Neurology	Unknown	Ovarian Cancer	Addenbrooke's Hospital, Cambridge, United Kingdom	https://clinicaltrials.gov/ct2/show/NCT03526809
Investigation of Differential Biology of Benign and Malignant Renal Masses Using Advanced Magnetic Resonance Imaging Techniques (IBM-Renal)	Recruiting	Kidney Cancer	University of Cambridge, United Kingdom	https://clinicaltrials.gov/study/NCT06016075
Hyperpolarized Carbon C 13 Pyruvate in Diagnosing Glioma in Patients with Brain Tumors	Recruiting	Primary Brain Neoplasm	M D Anderson Cancer Center, Houston, Texas, United States	https://clinicaltrials.gov/ct2/show/NCT03830151
HP Pyruvate MRI in Cancers (HC-MRI)	Recruiting	Clinical Tumor Diagnosis	University of Maryland, Baltimore, United States	https://clinicaltrials.gov/study/NCT05697406
Utility of Hyperpolarized 13C-pyruvate Metabolic Magnetic Resonance Imaging	Recruiting	Prostate Cancer	University of Maryland, Baltimore, United States	https://clinicaltrials.gov/study/NCT04698564
Hyperpolarized 13C-pyruvate Metabolic MRI With Infiltrating Gliomas	Recruiting	Glioma	University of Maryland, Baltimore, United States	https://clinicaltrials.gov/study/NCT04772456
Hyperpolarized 13C Pyruvate MRI for Early Immune Evaluation in Cervical Cancer Patients at Baseline and CCRT Therapy	Recruiting	Cervical Cancer	Chang Gung Memorial Hospital, Taiwan	https://clinicaltrials.gov/study/NCT04951921
Hyperpolarized 13C MRI for Cancer Immunotherapy (DNPSPIO)	Not yet Recruiting	Cervical, Endometrial, Ovarian Cancers	Chang Gung Memorial Hospital, Taiwan	https://clinicaltrials.gov/study/NCT05805358
13C Pyruvate DNP MR Spectroscopy for Lymphoma Treatment Response Assessment	Not yet Recruiting	Lymphoma	Chang Gung Memorial Hospital, Taiwan	https://clinicaltrials.gov/study/NCT05600361

Title	Status	Disease	Institution	Link
Hyperpolarized Carbon C 13 Pyruvate Magnetic Resonance Spectroscopic Imaging in Predicting Treatment Response in Patients with Prostate Cancer	Active, not recruiting	Prostate Adenocarcinoma PSA Level Greater Than Ten Stage IIB Prostate Cancer AJCC v8Stage III AJCC v8Stage IIIA AJCC v8Stage IIIB AJCC v8Stage IIIC AJCC v8Stage IV AJCC v8Stage IVA AJCC v8Stage IVB AJCC v8	M D Anderson Cancer Center, Houston, Texas, United States	https://clinicaltrials.gov/ct2/show/NCT03581500
Development and Evaluation of a Quantitative HP MRI for Clinical Prostate Cancer Exam	Active, not recruiting	Prostate Adenocarcinoma	M D Anderson Cancer Center, Houston, Texas, United States	https://clinicaltrials.gov/study/NCT04286386
An Investigational Scan (hpMRI) for Monitoring Treatment Response in Patients with Thyroid Cancer and Other Malignancies of the Head and Neck Undergoing Radiation Therapy and/or Systemic Therapy	Recruiting	Thyroid Carcinoma	M D Anderson Cancer Center, Houston, Texas, United States	https://clinicaltrials.gov/study/NCT04589624
Study Using Hyperpolarized 13C-Pyruvate Magnetic Resonance Spectroscopic Imaging in Patients with Pancreatic Cysts Undergoing Surgical Resection	Recruiting	Pancreatic disease	M D Anderson Cancer Center, Houston, Texas, United States	https://clinicaltrials.gov/study/NCT05873699
Hyperpolarized Carbon C 13 Pyruvate in Diagnosing Glioma in Patients with Brain Tumors	Recruiting	Primary Brain Neoplasm	M D Anderson Cancer Center, Houston, Texas, United States	https://clinicaltrials.gov/study/NCT03830151
Characterization of Hyperpolarized Pyruvate MRI Reproducibility	Recruiting	Malignant Solid Tumors	Memorial Sloan Kettering Cancer Center, New York, New York, United States	https://ClinicalTrials.gov/show/NCT02421380
Characterization of Hyperpolarized Pyruvate MRI Reproducibility	Recruiting	Malignant Solid Tumors	Memorial Sloan Kettering Cancer Center, New York, New York, United States	https://clinicaltrials.gov/ct2/show/NCT02421380

Title	Status	Disease	Institution	Link
A Study of [13C]Pyruvate as an Imaging Agent for Magnetic Resonance Imaging in Healthy Volunteers	Enrolling by Invitation	Healthy Volunteers	Memorial Sloan Kettering Cancer Center, New York, New York, United States	https://clinicaltrials.gov/study/NCT05041166
Hyperpolarized Carbon C 13 Pyruvate Magnetic Resonance Spectroscopic Imaging in Detecting Lactate and Bicarbonate in Participants with Central Nervous System Tumors	Copleted	Malignant Central Nervous System Neoplasm, Metastatic Malignant Neoplasm in the Central Nervous System	Stanford University School of Medicine, Palo Alto, California, United States	https://clinicaltrials.gov/ct2/show/NCT03565367
Metabolic Imaging of the Heart Using Hyperpolarized (13C) Pyruvate Injection	Recruiting	Hypertension Hypertrophy	Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada	https://ClinicalTrials.gov/show/NCT02648009
Multiparametric MRI for Prostate Cancer Localization and Characterization Using Hyperpolarized Pyruvate (13C) Injection	Withdrawn	Prostatic Neoplasms	Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada	https://ClinicalTrials.gov/show/NCT02647983
Study to Evaluate the Feasibility of 13-C Pyruvate Imaging in Breast Cancer Patients Receiving Neoadjuvant Chemotherapy	Withdrawn (Funding Completed)	Breast Cancer	Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada	https://ClinicalTrials.gov/show/NCT03121989
Role of Hyperpolarized 13C-Pyruvate MR Spectroscopy in Patients with Intracranial Metastasis Treated With (SRS)	Recruiting	Brain Metastases	Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada	https://ClinicalTrials.gov/show/NCT03324360
Hyperpolarized Carbon-13 Imaging of Metastatic Prostate Cancer	Terminated	Prostate Cancer	Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada	https://ClinicalTrials.gov/show/NCT02844647
Hyperpolarized 13C MR Imaging of Lactate in Patients with Locally Advanced Cervical Cancer (LACC) Cervical Cancer	Terminated	Uterine Cervical Neoplasms	Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada	https://ClinicalTrials.gov/show/NCT03129776
Hyperpolarized 13C-Pyruvate MRI Study	Unknown	Cancer, Cardiovascular Diseases	University College London, London, United Kingdom	https://clinicaltrials.gov/ct2/show/NCT03687645
Novel MRI Assessment of Prostate Cancer VALIDATE-PRO (VALIDATE-PRO)	Unknown	Prostate Cancer	University College London, London, United Kingdom	https://clinicaltrials.gov/study/NCT05017181

Title	Status	Disease	Institution	Link
Simultaneous Hyperpolarized [1-13C]Pyruvate and 18F-FDG PET/MRS in Cancer Patients	Enrolling by Invitation	Breast Cancer and Neuroendocrine tumors	Rigshopitalet, Copenhagen, Denmark	https://clinicaltrials.gov/study/NCT05396118
Pilot Study of Safety and Toxicity of Acquiring Hyperpolarized Carbon-13 Imaging in Children with Brain Tumors	Completed	Pediatric Brain Tumors	University of California San Francisco Helen Diller Family Comprehensive Cancer Center, San Francisco, California, United States	https://ClinicalTrials.gov/show/NCT02947373
Hyperpolarized Pyruvate Injection in Subjects with Prostate Cancer	Completed	Prostate Cancer	University of California San Francisco, San Francisco, California, United States	https://ClinicalTrials.gov/show/NCT01229618
Hyperpolarized C-13 Pyruvate as a Biomarker in Patients with Advanced Solid Tumor Malignancies	Terminated	Prostate Cancer	University of California, San Francisco, San Francisco, California, United States	https://ClinicalTrials.gov/show/NCT02913131
A Pilot Study of (MR) Imaging with Pyruvate (13C) to Detect High Grade Prostate Cancer	Recruiting	Prostate Cancer	University of California, San Francisco, San Francisco, California, United States	https://ClinicalTrials.gov/show/NCT02526368
Magnetic Resonance (MR) Imaging with Hyperpolarized Pyruvate (HP) (13C) in Castration-Resistant Prostate Cancer	Terminated	Prostate Cancer	University of California, San Francisco, San Francisco, California, United States	https://ClinicalTrials.gov/show/NCT02911467
MRI with C13 Pilot Study Prostate Cancer	Terminated	Prostate Cancer	University of California, San Francisco, San Francisco, California, United States	https://ClinicalTrials.gov/show/NCT02450201
Hyperpolarized Carbon-13 (13C) Pyruvate Imaging in Patients with Glioblastoma	Active, No Recruiting	Glioblastoma Multiforme (GBM)	University of California, San Francisco, San Francisco, California, United States	https://clinicaltrials.gov/ct2/show/NCT04019002

Title	Status	Disease	Institution	Link
Hyperpolarized Pyruvate (13C) MR Imaging in Monitoring Patients with Prostate Cancer on Active Surveillance	Recruiting	Prostate Adenocarcinoma	University of California, San Francisco, San Francisco, California, United States	https://clinicaltrials.gov/ct2/show/NCT03933670
Hyperpolarized Imaging in Diagnosing Participants with Glioma	Recruiting	Glioma	University of California, San Francisco, San Francisco, California, United States	https://clinicaltrials.gov/ct2/show/NCT03739411
Hyperpolarized 13C Pyruvate as a Biomarker in Advanced Solid Tumors	Recruiting	Advanced Solid Tumor	University of California, San Francisco, San Francisco, California, United States	https://clinicaltrials.gov/study/NCT05599048
Hyperpolarized 13C Pyruvate MRI for Treatment Response Assessment in Pancreatic Ductal Adenocarcinoma	Terminated	Pancreatic Ductal Adenocarcinoma	University of California, San Francisco, San Francisco, California, United States	https://clinicaltrials.gov/study/NCT04565327
Feasibility of Acquiring Hyperpolarized Imaging in Patients with Primary CNS Lymphoma	Recruiting	CNS Lymphoma	University of California, San Francisco, San Francisco, California, United States	https://clinicaltrials.gov/study/NCT04656431
Feasibility of Acquiring Hyperpolarized Imaging in Patients with Meningioma	Recruiting	Meningioma	University of California, San Francisco, San Francisco, California, United States	https://clinicaltrials.gov/study/NCT06014905
Serial MR Imaging and MR Spectroscopic Imaging for the Characterization of Lower Grade Glioma	Recruiting	Low Grade Glioma	University of California, San Francisco, San Francisco, California, United States	https://clinicaltrials.gov/study/NCT04540107
Magnetic Resonance Imaging (MRI) With Hyperpolarized Pyruvate (13C) as Diagnostic Tool in Advanced Prostate Cancer	Recruiting	Prostate Cancer	University of California, San Francisco, San Francisco, California, United States	https://clinicaltrials.gov/study/NCT04346225

Title	Status	Disease	Institution	Link
Hyperpolarized 13C Pyruvate MRI Scan in Predicting Tumor Aggressiveness in Patients With Renal Tumors	Recruiting	Kidney Neoplasm, Renal Cell Cancer	University of California, San Francisco, San Francisco, California, United States	https://clinicaltrials.gov/study/NCT04258462
Effect of Fatty Liver on TCA Cycle Flux and the Pentose Phosphate Pathway	Enrolling by invitation	Fatty Liver	UT Southwestern - Advanced Imaging Research Center, Dallas, Texas, United States	https://clinicaltrials.gov/ct2/show/NCT03480594
Imaging of Traumatic Brain Injury Metabolism Using Hyperpolarized Carbon-13 Pyruvate	Enrolling by invitation	Traumatic Brain Injury	UT Southwestern – Advanced Imaging Research Center, Dallas, Texas, United States	https://clinicaltrials.gov/ct2/show/NCT03502967
Using Hyperpolarized [1-13C]Pyruvate to Detect Cardiotoxicity (HPCardiotox)	Enrolling by invitation	Breast Neoplasms	UT Southwestern – Advanced Imaging Research Center, Dallas, Texas, United States	https://clinicaltrials.gov/ct2/show/NCT03685175
Metabolic Characteristics of Sarcoma In Vivo Using HP 13-C Magnetic Resonance Spectroscopic Imaging (MRSI)	Withdrawn	Sarcoma, Soft Tissue	UT Southwestern – Advanced Research Center, Dallas, Texas, United States	https://clinicaltrials.gov/ct2/show/NCT03759704
Metabolic Characteristics of Brain Tumors Using Hyperpolarized Carbon-13 Magnetic Resonance Spectroscopic Imaging (MRSI)	Recruiting	Brain Tumor Adult	UT Southwestern Medical Center, Dallas, Texas, United States	https://ClinicalTrials.gov/show/NCT03067467
UTSW HP [13-C] Pyruvate Injection in HCM	Recruiting	Cardiomyopathy, Hypertrophic	UT Southwestern Medical Center - Advanced Imaging Research Center, Dallas, Texas, United States	https://ClinicalTrials.gov/show/NCT03057002
Imaging Oxidative Metabolism and Neurotransmitter Synthesis in the Human Brain	Enrolling by invitation	Brain Cancer	UT Southwestern Medical Center - Advanced Imaging Research Center, Dallas, Texas, United States	https://clinicaltrials.gov/ct2/show/NCT03849963

Title	Status	Disease	Institution	Link
Hyperpolarized Carbon 13-Based Metabolic Imaging to Detect Radiation-Induced Cardiotoxicity	Active, not recruiting	Left-Sided Breast Cancer	UT Southwestern Medical Center, Department of Radiation Oncology, Dallas, Texas, United States	https://clinicaltrials.gov/ct2/show/NCT04044872
Feasibility Study Using Imaging Biomarkers in Lung Cancer	Recruiting	Lung Cancer	UT Southwestern Medical Center - Advanced Imaging Research Center, Dallas, Texas, United States	https://clinicaltrials.gov/study/NCT02095808

Table 3. Current Trials listed on clinicaltrialsregister.eu

Title	Status	Disease	Institution	Link
Early detection of effects of chemotherapy in pancreatic cancer patients – a study using MR-hyperpolarization scanning based on hyperpolarized Pyruvate (13C) injection	Recruiting	Pancreatic Cancer	Aarhus University, Dept. Clinical Medicine, Aarhus, Denmark	https://www.clinicaltrialsregister.eu/ctr-search/trial/2016-004491-22/DK
Clinical and pathophysiological aspects of visualization of metabolic flux in the failing human heart using hyperpolarized [1-13C]-pyruvate cardiac magnetic resonance	Recruiting	Chronic Heart Failure	Aarhus University, Dept. Cardiology, Aarhus, Denmark	https://www.clinicaltrialsregister.eu/ctr-search/trial/2018-003533-15/DK
Metabolic imaging of patients with mycosis fungoides using hyperpolarized 13C-Pyruvate magnetic resonance imaging – A feasibility study	Prematurely Ended	Mycosis fungoides	Aarhus University, Dept. Dermatology, Aarhus, Denmark	https://www.clinicaltrialsregister.eu/ctr-search/trial/2018-001656-35/DK
Metabolic MRI with hyperpolarized pyruvate in long-term COVID19 patients	Prematurely ended	COVID-19	Aarhus University, Dept. Dermatology, Aarhus, Denmark	https://www.clinicaltrialsregister.eu/ctr-search/trial/2021-001031-72/DK
MRI with Hyperpolarized Pyruvate in Glioblastoma – a Phase II Study	Trial now transitioned	Glioblastoma	Aarhus University, Dept. Dermatology, Aarhus, Denmark	https://www.clinicaltrialsregister.eu/ctr-search/trial/2020-000310-15/DK
Early detection of hepatocellular carcinoma by Hyperpolarized [1-13C]pyruvate MRI	Trial now transitioned	Primary liver cancer	Aarhus University, Dept. Dermatology, Aarhus, Denmark	https://www.clinicaltrialsregister.eu/ctr-search/trial/2021-000863-56/DK

Title	Status	Disease	Institution	Link
MRI of neurometabolic impairment in ALS and TIA using hyperpolarized pyruvate	Recruiting	TIA, Stroke, ALS	Aarhus University, Dept. Dermatology, Aarhus, Denmark	https://www.clinicaltrialsregister.eu/ctr-search/trial/2020-000352-36/DK
Chronic kidney disease – imaging the metabolic derangements with ultra-sensitive MRI	Trial now transitioned	Chronic kidney disease	Aarhus University, Dept. Dermatology, Aarhus, Denmark	https://www.clinicaltrialsregister.eu/ctr-search/trial/2021-002551-11/DK

Table 4. Current Trials listed on WHO and ORCID

Title	Status	Disease	Institution	Link
Cardiac Magnetic Resonance for the Prediction and Diagnostics of Heart Failure	Recruiting	Heart Failure	ETH and University Zurich, Institute for Biomedical Engineering, Zurich, Switzerland	http://apps.who.int/trialsearch/Trial2.aspx?TrialID=DRKS00016614

3. INTRODUCTION

3.1 Investigational Medicinal Product

Pyruvate Injection is a sterile solution for intravenous injection. In addition to the active pharmaceutical ingredient, pyruvate, the solution for injection will contain AH111501 (Electron Paramagnetic Agent; EPA), TRIS and Na_2EDTA and have a pH within the physiological range.

In ^{13}C MR imaging studies that follow the previous (GE-101-001 and GE-101-003) safety and tolerability studies in young and elderly volunteers, the investigational medicinal product is Hyperpolarized Pyruvate (^{13}C) Injection, containing spin-polarized ("hyperpolarized") [1-^{13}C]pyruvate. ^{13}C is a stable, non-radioactive isotope of carbon. [^{13}C]pyruvate has the same chemical characteristics as pyruvate. The only difference is that one or two of the carbon nuclei have been replaced by a ^{13}C-nucleus, which has a magnetic moment and can be hyperpolarized. AH111501 (EPA), which is a stable trityl radical, is an excipient, and enables the hyperpolarization by Dynamic Nuclear Polarization, a novel polarization technology.

As all isotopic labeling positions of [^{13}C]pyruvate have the same chemical characteristics as pyruvate, they will be metabolized in the same way and have the same safety profile. Formulations of Pyruvate Injection made with different [^{13}C] isotopic labeling positions and unlabeled pyruvate are expected to have the same safety profiles as the impurity profiles of the formulations are similar. The safety profiles of various formulations of pyruvate administered at high doses by slow infusion are documented in published literature (see Section 6, Effects in Humans). In the 2 completed phase 1 studies (GE-101-001 and GE-101-003) and the planned safety and imaging study, the maximum dose of pyruvate is much lower. However, owing to the short relaxation time of the hyperpolarized state, the time window in which [^{13}C]pyruvate can be imaged by ^{13}C MR imaging will be short (approximately 1.5 minutes post-injection). Therefore, Pyruvate Injection is administered as a bolus injection at a rate of 5 ml/s; as such, pyruvate is administered at a much higher rate than in the published 'infusion' studies. This mode of administration was well tolerated in studies GE-101-001 and GE-101-003 (see Section 6, Effects in Humans).

3.2 Research Rationale

Pyruvate is an important product of glycolysis and can be converted to various metabolites via three essential biochemical pathways associated with pyruvate dehydrogenase (PDH), lactate dehydrogenase (LDH) and alanine transaminase (ALT). Figure 1 shows a simplified diagram of the biochemical pathways that follows the metabolisms of [1-13C]pyruvate. Explicitly, [1-13C]pyruvate will be reduced by the NADH produced in the pathway to generate [1-13C]lactate, in the reaction catalyzed by the enzyme LDH undergoes transamination with glutamate to form [1-13C]alanine, in the reaction catalyzed by the enzyme ALT and involves the irreversible decarboxylation of [1-13C]pyruvate to hyperpolarized [13C]CO_2 in the reaction catalyzed by the mitochondrial enzyme PDH. The [13C]carbon dioxide released is subsequently interconverted with [13C]bicarbonate in the reaction catalyzed by carbonic anhydrase. These metabolites can be readily detected by 13C MRS and 13C MRI. Thus, hyperpolarized [1-13]C pyruvate has the potential to assess the flux through these metabolic pathways, which normally reflect the metabolic status of diseased tissues and their therapeutic responses to treatment. The biochemical pathway depicted is identical for all labeling positions of [^{13}C]pyruvate. Figure 2 shows an example case of prostate cancer.

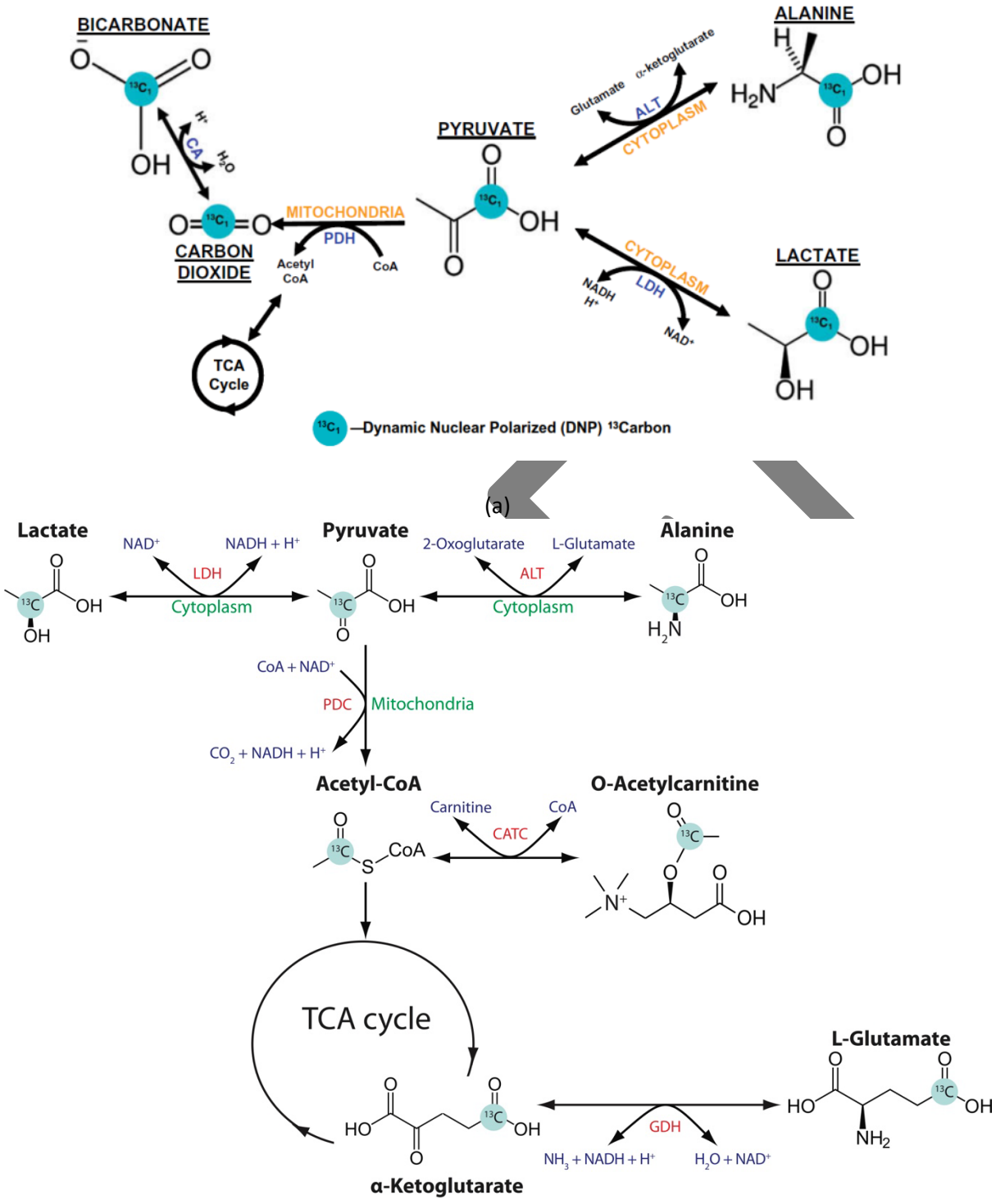


Figure 1. Simplified diagram of the biochemical pathways taken by [1-13C]pyruvate and [2-13C]pyruvate, respectively, that are visible with the hyperpolarized MR spectroscopy techniques discussed here. The position of the hyperpolarized 13C nucleus is shown in all metabolites with the 13C symbol. LDH = lactate dehydrogenase. PDH, pyruvate dehydrogenase complex. ALT, alanine transaminase. CA, carbonic anhydrase.

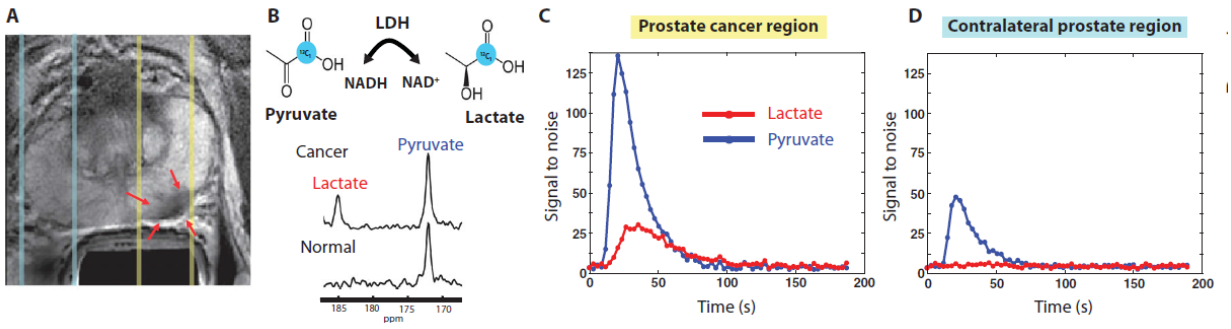


Figure 2. Images are from a representative patient with a current PSA of 12.2 ng/ml, a small volume of biopsy-proven Gleason grade 4 + 3 prostate cancer in the left mid gland, and who received the lowest dose (0.14 ml/kg) of hyperpolarized [1-13C]pyruvate. (A) Axial T2-weighted image showing slices (dashed lines) obtained from 1D spectral localization. (B) Flux of [1-13C]pyruvate to [1-13C]lactate catalyzed by LDH (top). Dynamic 13C spectra were obtained from the same patient in (A) at 36 s after injection of hyperpolarized [1-13C]pyruvate (bottom). (C) Plot of 1D localized dynamic hyperpolarized pyruvate and lactate data from the slice that overlapped the region of prostate cancer. (D) Plot of 1D localized dynamic hyperpolarized pyruvate and lactate data from the slice that overlapped a contralateral region of the prostate.

In healthy tissues, pyruvate is preferentially converted in the mitochondria to acetyl-CoA and carbon dioxide via PDH. This is followed by entrance into the tricarboxylic acid (TCA) cycle to eventually provide cellular energy as ATP, known as oxidative phosphorylation (OXPHOS) in the mitochondria. The conversion to alanine via ALT is known to be predominant in certain healthy tissues such as liver and sometimes muscle. These preferring pathways are highly dynamic that they are readily altered under perturbations of therapeutic stress and/or tissue microenvironment conditions. As an example, without oxygen, or if the TCA cycle is running at full capacity, healthy tissue is forced to maintain energy needs through an alternate pathway that converts pyruvate to lactate via LDH and oxidizes NADH to NAD+. The NAD+ is then available to aid in further glycolytic conversion of glucose to pyruvate, providing cellular energy as ATP.

In diseased tissues, the metabolism of pyruvate is likely to be shifted away from its normal flux patterns. In oncology, most cancer cells predominantly produce energy by a high rate of glycolysis followed by lactic acid fermentation in the cytosol, known as aerobic glycolysis, or the Warburg effect, rather than a comparatively low rate of glycolysis followed OXPHOS in the mitochondria. Some cancer cells also show changes in transaminase activity, suggesting pyruvate-to-alanine exchange may be enhanced or suppressed, depending on tumor type. In heart, the cardiac metabolism switches rapidly among a wide variety of substrates to supply acetyl-CoA, which typically involves four groups of reactions, or pathways to support this process: β-oxidation, glycolysis, the pentose phosphate pathway and the citric acid cycle. The PDH enzyme complex is a fundamental determinant of the relative contributions of glucose and fatty acid oxidation to ATP production in the heart, and its activity is correlated with the status of the heart disease such as myocardial ischemia (MI), diabetic cardiomyopathy, etc. This metabolic shift also occurs in many other diseases, i.e., an increase of lactic acid in inflammatory arthritis and ischemic tissues due to increase energy demands an elevated hepatic ALT activity in liver diseases. Also, the metabolic status can be a marker for the functions of certain organs, i.e., an elevated increased oxidative phosphorylation

occurs in brown adipose tissue; metabolic status of placenta reflecting its role in mediating interactions & nutrient transport between mother and fetus. The metabolism of pyruvate in diseased tissues can be altered substantially via various therapeutic treatments, including modulation of major signaling pathways such as PI3K/Akt/mTOR, MEK, VEGFR, KRas, Myc, p53,etc., inhibition of metabolic pathways for PDH, LDH, HK2, GLS1 etc., immunotherapy, radiation therapy and chemotherapy.

4. PHYSICAL, CHEMICAL AND PHARMACEUTICAL PROPERTIES AND FORMULATION

4.1 Name and Description of Investigational Medicinal Product

Hyperpolarized Pyruvate (^{13}C) Injection (drug product) is a sterile solution for intravenous injection. The preparation of Hyperpolarized Pyruvate (^{13}C) Injection is performed by an automated equipment known as SPINlab™. For each patient dose, SPINlab utilizes a fluid path that contains the following three active pharmaceutical ingredients:

- Mixture of [^{13}C]pyruvic acid and 15 mM AH111501 sodium salt
- Sterile Water for Injection (WFI)
- TRIS/EDTA buffer solution

Assembly of the parts and components and filling of the fluid path yields a Kit for the preparation of Hyperpolarized (^{13}C) Injection. The Kit and the drug product are prepared at the clinical site according to local regulations. The SPINlab™ is located as close as feasible to the MRI imaging instrument.

At the time of the study, the cryovial part of the fluid path, which contains the mixture of [^{13}C]pyruvic acid and 15 mM AH111501 sodium salt, is lowered into the polarizer of SPINlab™ and polarized for approximately 60 minutes at 1.2 K. After polarization, the mixture of [^{13}C]pyruvic acid and 15 mM AH111501 sodium salt is flushed out of the cryovial with heated and pressurized sterile WFI into the tubing of the path and is passed through a coarse (40 µm average pore size) and a fine (13 µm average pore size) filter assembly made of a ultra-high molecular weight polyethylene (UHMWPE) for removal of AH111501. The solution is then mixed with the TRIS/EDTA buffer solution contained in a receiver vessel. An aliquot of the buffered drug product solution is tested by the automated quality control instrument (QC System) of SPINlab™ and complemented by alternate tests if needed. The drug product solution is then passed through a sterilizing filter (0.22 µm) before entering the syringe/drug product container. In most cases, the sterilizing filter is tested for integrity by a subsequent bubble point test. Based on the results from the testing, the final release authorization and approval for administration to humans will be given by appropriate personnel. Samples are further tested for sterility and endotoxin after drug product administration.

A consensus view related to the production and quality control of [13C] pyruvate for MRI human studies was published by Peder Larson et al in Magnetic Resonance in Medicine titled “Current methods for hyperpolarized [1-13C] pyruvate MRI human studies.

The T1 relaxation time (the time constant for the hyperpolarization decay) of [^{13}C]pyruvate after dissolution is about 70s.

4.2 Pharmaceutical properties and formulation

Hyperpolarized Pyruvate ^{13}CInjection is a sterile solution for intravenous administration. The pH of the solution for injection is in the physiological range typically between pH 6.5 and 8.5, and the osmolality is ~500 mOsm/kg. The solution for injection contains 250 mM [^{13}C]pyruvate , 100 mM TRIS, 0.1 mg/mL Na_2EDTA and no more than 3.0 µM AH 111501.

4.3 Storage and use

Due to the rapid, post-compounding decay of hyperpolarization, the Hyperpolarized Pyruvate (^{13}C) Injection should be administered to the subject who is to undergo MR imaging as soon as possible

after compounding and quality control.

The Kit for the preparation of Hyperpolarized (^{13}C) Injection can be assembled, filled and stored, typically at -20 $\pm$ 5 ^{o}C, based on additional supporting stability data.

5. NONCLINICAL STUDIES

A range of nonclinical studies has been undertaken as part of the development program which was designed to support the planned early clinical studies. These studies used non-C-13 enriched pyruvate and impurities, [1-^{13}C]pyruvate , hyperpolarized [1-^{13}C]pyruvate , and [1-^{14}C]pyruvate, as appropriate. The program comprised:

- A pharmacodynamic study in the healthy dog using hyperpolarized [1-^{13}C]pyruvate.
- Two biodistribution studies in rats using [1-^{13}C]pyruvate test articles spiked with [1-^{14}C]pyruvate.
- Safety pharmacology studies assessing effects on 1) the hERG channel, 2) the cardiovascular system in the conscious and anesthetized dog, 3) CNS in the conscious rat and dog, and 4) the respiratory system in the conscious dog. In these studies, two [1-^{13}C]pyruvate test articles and various pyruvate test articles were used.
- Toxicology studies including 1) expanded acute toxicity in the rat and dog, 2) studies of genetic toxicology (*in vitro* and *in vivo*) using [1-^{13}C]pyruvate test articles, and 3) a study of local tolerance using a pyruvate test article
- Qualification studies of the three potential impurities: AH112623 (parapyruvate), AH112615 (reaction-product of pyruvic acid and TRIS) and AH113462 (lactone). The studies include 1) expanded acute toxicity in the rat, and 2) studies of genetic toxicology (*in vitro* and *in vivo*)
- Studies of the novel ingredient AH111501 including 1) the hERG assay and 2) *in vitro* genetic toxicology.

[^{13}C]pyruvate and pyruvate have the same chemical characteristics and will therefore have the same safety profile, as the impurity profiles of the test articles are similar.

In one of the pharmacokinetic studies and in all GLP safety pharmacology and toxicology studies the pyruvate or [1-^{13}C]pyruvate test articles contained AH111501.

The injection dose is 0.43 mL/kg of a drug product test article having a pyruvate concentration between 220 and 280 mM, no more than 3.0 µM residual AH 111501 and in a TRIS/EDTA buffer of pH 6.7 to 8.0. The recommended clinical injection rate is 5 mL/s.

5.1 Nonclinical Pharmacology

5.1.1 Pharmacodynamics

5.1.1.1 Brief Summary

Intravenous administration of hyperpolarized [^{13}C]pyruvate enables real-time detection of metabolism by magnetic resonance spectroscopic imaging (MRSI). The signal from a given tissue depends on the dose administered, the perfusion of the tissue and the rate of formation of the metabolites. The relative levels of the metabolites depend on the energy needs of the specific tissue. With this technique it is expected that cancers with a high metabolic rate can be distinguished from surrounding healthy tissue by measuring the formation of [^{13}C]lactate.

Based on high-resolution magic angle spinning (HR-MAS) spectroscopic analysis of human prostate tissue samples, it is expected that the overall glycolytic activity in prostate cancer is sufficiently high when compared to healthy prostate tissue that this cancer can be detected by [^{13}C]pyruvate

imaging. Nonclinical imaging studies in the transgenic adenocarcinoma of mouse prostate (TRAMP) mouse support this hypothesis. Studies in lymphoma-bearing mice have demonstrated that the technique is sensitive, as it can detect changes in tumor metabolism 24 h after chemotherapy. Studies in healthy dogs indicate that the technique can be scaled for use in man.

Safety studies of the cardiovascular effects of the hyperosmotic pyruvate test article in the conscious and anesthetized dog demonstrated that the test article causes vasodilation in a dose-dependent manner followed by compensatory tachycardia and a slight increase in cardiac output. The test article was found to have no measurable effect on the central nervous system in the conscious rat and dog.

The core-battery of safety pharmacology studies was performed according to the ICH S7A and S7B guidelines.

5.1.1.2 Primary Pharmacodynamics, Evaluation of Performance

5.1.1.2.1 Quantitative Analysis of Prostate Metabolites Using 1H HR-MAS Spectroscopy [Swanson et al. 2006]

Samples of excised prostate were selected by visually aligning presurgical MRI/3D-MRSI data with tissue sections to identify the section corresponding to the most metabolically and anatomically abnormal region. One piece of tissue was then cut from this section, and another was cut from a contralateral benign-appearing region. High-resolution magic angle spinning (HR-MAS) spectroscopic analysis of 60 samples obtained from 20 patients demonstrated significantly higher concentrations of lactate in prostate cancer tissues (70±27 mmol/kg) than healthy glandular (47±17 mmol/kg, $p<0.01$) and stromal tissues (45±19 mmol/kg $p<0.01$). There was no measurable glucose in the tissues, suggesting the complete anaerobic conversion of glucose to lactate and alanine during the surgical and tissue harvesting procedures.

5.1.1.2.2 Evaluation of Lactate and Alanine as Metabolic Biomarkers of Prostate Cancer Using 1H HR-MAS Spectroscopy of Biopsy Tissues [Tessem et al. 2008]

Samples of snap-frozen transrectal ultrasound (TRUS)-guided prostate biopsy tissues were analyzed by HR-MAS spectroscopy for concentration of lactate. A total of 130 biopsies from 82 previously untreated patients were analyzed and out of these, 32 biopsies were considered unusable due to spectral contamination from lipids and/or topical anesthetic based on both 1D and 2D spectral findings. Samples included in the study then underwent a complete pathologic assessment of the core. Eighty-two of the biopsies were benign and 16 contained prostate cancer. There was a highly significant increase in lactate concentrations ($P < 0.0001$) in prostate cancer vs. benign prostate biopsy tissues. The average lactate concentration was 1.59 ± 0.61 mmol/kg in cancer tissues ($N = 16$) and 0.61 ± 0.28 mmol/kg in benign tissues ($N = 82$). Out of the 82 benign biopsies, only 15 samples had individual lactate concentrations overlapping with the concentrations observed in malignant samples. The significant increase in the concentration of lactate in biopsy samples containing as little as 5% cancer, and the minimal overlap of lactate concentrations between benign and malignant biopsies suggest that lactate will be a useful biomarker that could be utilized in hyperpolarized ^{13}C MRSI staging exams of prostate cancer patients.

5.1.1.2.3 Hyperpolarized ^{13}C Lactate, Pyruvate, and Alanine – Non-Invasive Biomarkers for Prostate Cancer Detection and Grading [Albers et al. 2008]

Four TRAMP mice (21-28 wks of age) with tumors histologically classified as low grade and three (27-40 wks of age) with high grade tumors and lymph node metastasis were imaged a total of six and five times, respectively, following administration of hyperpolarized [1-^{13}C]pyruvate (28 µmol/mouse). In addition, five normal mice were imaged a total of seven times as part of the study. Within one week, the mice were dissected, and the tumors were histologically examined. The studies showed that mice with high grade or poorly differentiated prostate cancer had higher levels of lactate and lower levels of pyruvate than mice with low grade or well differentiated prostate cancer (Figure 3). This provides evidence for the concept of using Hyperpolarized (^{13}C) Injection in humans to assess the grade of prostate cancer.

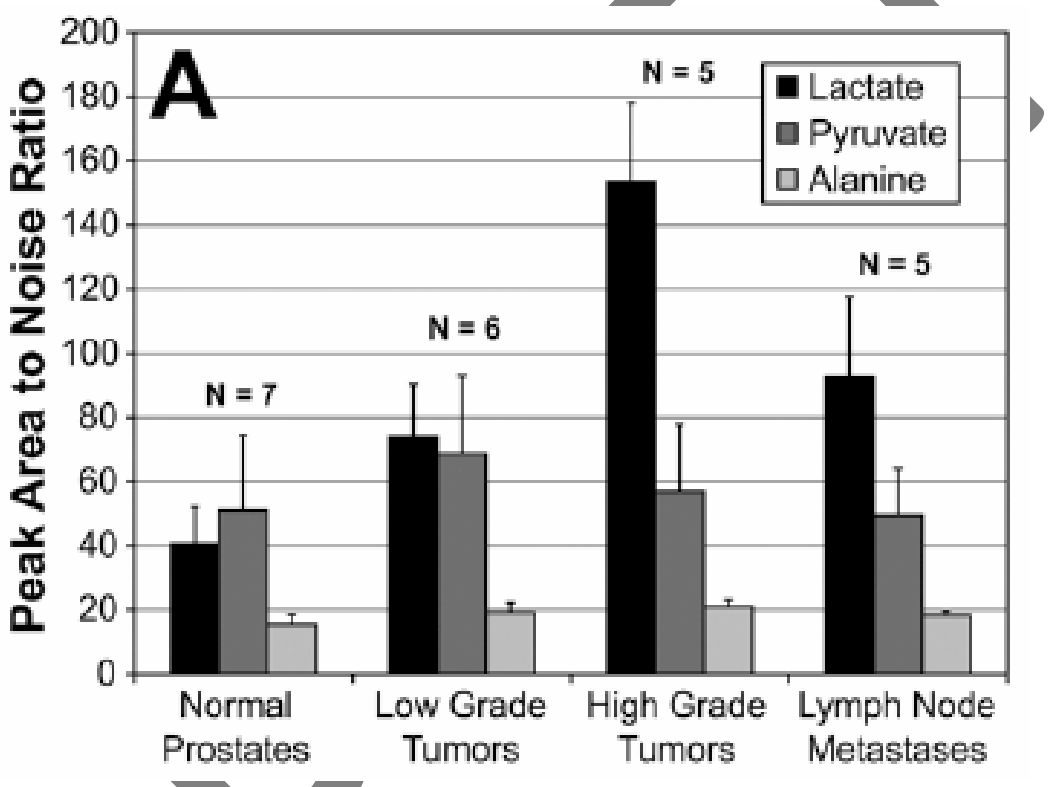


Figure 3. Hyperpolarized ^{13}C metabolites in the pathologically defined groups. The lactate peak area signal-to-noise ratio (SNR) values were statistically different for all four groups, except that low grade tumors were not different from lymph node metastases ($p < 0.05$).

5.1.1.2.4 Detecting Tumor Response to Treatment Using Hyperpolarized ^{13}C Magnetic Resonance Imaging and Spectroscopy [Day et al. 2007]

Mice with lymphoma tumors induced by subcutaneous implantation of the mouse tumor cell- line EL-4 were imaged following administered hyperpolarized [1-^{13}C]pyruvate (15 µmol/mouse). In this

model relatively low levels of [1-^{13}C]lactate were formed in the muscle and liver when compared to the tumor. In animals imaged 24 h after treatment with the chemotherapeutic agent etoposide (67 mg/mouse), data revealed a significant reduction of conversion of pyruvate to lactate (p=0.01). Measurements of treated tumors indicated that the reduction in part was due to loss of NAD(H) and decreased lactate-dehydrogenase activity and lactate concentration. Even though the EL-4 model is sensitive to treatment, as is apparent from the reduction in tumor volume of 17% at the imaging time point, the data suggest the imaging technique is very sensitive.

5.1.1.2.5 GE study number: B101059 Metabolic Imaging with Hyperpolarized 13C-Pyruvate in Prostate of Healthy Dogs Using a Second-Generation Endorectal Coil and Reduced Dose

The study was designed to provide a realistic test of the methodology to be applied in exploratory imaging studies in humans, as similar coil geometry and data acquisition parameters were used for the dogs as are being planned for humans. Specific goals of the study were to test the improved radiofrequency coils and pulse sequences being developed for applications using 250 mM Hyperpolarized Pyruvate (^{13}C) Injection in doses of 0.43 ml/kg or less, to determine the dynamics of arrival of the [1-^{13}C]pyruvate bolus, to evaluate the signal to noise ratio (SNR) of [1-^{13}C]pyruvate in healthy dog prostate, and to investigate whether its metabolic products could be observed at dose levels of one-quarter (0.36 ml/kg) and one-eighth (0.18 ml/kg) of a dose of 1.4 ml/kg previously demonstrated to be efficacious in the healthy dog (Study B101033).

The study demonstrated lactate and pyruvate signal to noise ratios of approximately 20:1 at both the 0.18 ml/kg and 0.36 ml/kg dose levels using the second-generation clinical coils and newly developed MRSI sequence. It was, however, noted that the choice of dose appears to be limited by tissue contrast to noise. Based on this, it was recommended using the highest dose acceptable from a safety perspective in clinical studies. Furthermore, it was evident that the pulse sequence must be tailored to suit the biology of the human prostate and this can only be accomplished in human subjects.

5.1.1.3 Imaging sequence development

Methods have been evaluated *in vitro*, and *in vivo* in normal rat kidney and in canine prostate to determine the most effective way to sample hyperpolarized [1-^{13}C]pyruvate metabolic images with a focus on lactate and pyruvate signal to noise and image quality. In addition, T_2 measurements were made in normal tissue and in TRAMP tumor tissue to estimate the impact of this key imaging parameter [Yen 2006].

Pulse sequences are being developed to achieve HP 13C MRSI at higher spatial & temporal resolution with reduced artifacts. These sequences include:

(1) Methods based on compressed sensing echo-planar spectroscopic imaging (EPSI) [Hu, et al. 2008, Hu, et al. 2010, Ohliger, et al. 2013], multi-band RF excitation EPSI [Larson, et al., 2008, Larson, et al., 2010, Larson, et al. 2011] or least- square CSI (LSCSI) [Reeder, et al, 2007];

(2) Methods based on spiral chemical shift imaging spCSI [Mayer, et al. 2006, Mayer, et al., 2009, Mayer, et al ., 2011], least- square spiral CSI [Levin, et al., 2007], cardiac-gated spiral CSI [Lau, et al., 2014], Iterative *D*ecomposition of water and fat with *E*cho *A*symmetry and *L*east-squares

estimation(IDEAL) spiral CSI [Wiesinger, et al., 2012];

(3) Methods based on single-shot MRSI with spatial-temporal encoding (SPEN) [Schmidt, et al., 2014], single voxel MRSI [Chen, et al., 2015];(

4) Method that can separate information such as metabolic activity decomposition-stimulated echo acquisition mode (MAD-STEAM) [Swisher, et al., 2014].

5.1.1.4 Summary and Conclusions for Evaluation of Performance

Based on the study in TRAMP mice it appeared feasible to use hyperpolarized [1-^{13}C]pyruvate metabolic imaging to distinguish cancerous prostate tissue from healthy tissue. During prostate cancer progression from early- to late-stage primary disease in the model there was a significant increase in pyruvate metabolism to lactate. These findings suggested that hyperpolarized [1-^{13}C]pyruvate metabolic imaging may be useful in diagnosing prostate cancer and in assessing stage and potentially aggressiveness of the cancer. The study in the lymphoma tumor model supported the ability of the technique to identify cancerous tissue and indicated that the technique may also become useful for assessing response to therapy. It should be noted that the two studies in mice were conducted using relatively high doses of hyperpolarized [1-^{13}C]pyruvate per kg bw. The metabolic imaging technique required dedicated imaging sequences and several nonclinical studies have been conducted to develop clinically useful sequences. The dog study demonstrated that good SNR can be achieved in the prostate of a healthy large animal at doses relevant for clinical studies, but also indicated that to develop optimal sequences for imaging of the human prostate clinical studies were required.

The above nonclinical imaging data demonstrated that it was feasible to perform MR metabolic imaging of prostate cancer using Hyperpolarized Pyruvate (^{13}C) Injection.

5.1.2 Secondary Pharmacodynamics

No specific studies have been performed to date.

5.1.3 Safety Pharmacology

In all nonclinical safety pharmacology studies except for the hERG assay, the test item was prepared by dissolving non-hyperpolarized [1-^{13}C]pyruvic acid or pyruvic acid, with or without AH111501, in a TRIS/EDTA dissolution medium, followed by heating to 80°C and cooling to 37°C. The preparation of the nonclinical test item was performed to mimic, as far as practically possible, the then intended preparation process for Hyperpolarized Pyruvate (^{13}C) Injection. Initially, a test article of 500 mM (44 mg/ml) pyruvate and 0.85 mg/ml AH111501 in 200 mM TRIS with 0.1 mg/ml Na_2EDTA and 360 mM NaOH was used in the nonclinical studies. Two non-GLP studies of the cardiovascular effects of rapid (5 ml/s) intravenous administration of the test article (without AH111501) demonstrated that the high osmolality (approx. 1000 mOsmol/kg) contributed to effects on the cardiovascular system. A test article of 250 mM (22 mg/ml) pyruvate and 0.43 mg/ml AH111501 in 100 mM TRIS with 0.1 mg/ml Na_2EDTA and 180 mM NaOH was used for the remaining studies. This test article had an osmolality of approx. 500 mOsmol/kg.

In several studies a test item where AH111501 had been removed by solid phase extraction following dissolution was used. This test item is referred to as “filtered test item” in the following

text. Filtered test item contained approx. 0.4 µM AH111501.

All test item test articles were used within 4 h after of dissolution.

5.1.3.1 In vitro assays

5.1.3.1.1. RCC Study 858776 (GE Study Number B101021) Na-pyruvate and AH111501: Effect on HERG-1 Tail Currents Recorded from Stably Transfected HEK 293 Cells (GLP)

hERG-1 tail currents elicited by a voltage protocol were recorded from stably transfected HEK 293 cells using the whole-cell patch-clamp technique. The test items were investigated at nominal concentrations of 7.35 mg/ml for AH111501 and an initial target concentration of 8 mg/ml for sodium pyruvate. A hyperosmolar control (22 mg/ml mannitol) and the reference item E-4031 were investigated in separate groups of cells. Vehicle effects were investigated in an additional group of cells. All recordings were performed at room temperature. hERG-1 tail current amplitudes at the end of the application were compared to the tail current amplitudes at the pre-treatment phase of the same cell (relative tail current).

Exposure levels of cells treated with sodium pyruvate could not be confirmed, as this test item was found not to be stable while frozen. The study had to be repeated at another test site. At this site the initial planned test concentration of 8 mg/ml sodium pyruvate could not be assayed due to non-specific membrane effects observed right after perfusion to the patch-clamped cell. The tail current inhibition present in these experiments was ascribed to an increase in the series resistance rather than an interaction of the test item with the hERG channel. Thus, sodium pyruvate was tested at a lower concentration of 4 mg/ml.

In the initial recordings a mean relative tail current of 93.8% was seen for the vehicle, and a mean relative tail current of 83.5% was observed in the cells treated with 7.35 mg/ml AH111501. The relative tail current amplitude of the cells treated with the reference item (100 nM E-4031) was 14.3%, supporting validity of the employed test system. In the second series of experiments a mean relative tail current of 101.0% was noted in the cells treated with 4 mg/ml sodium pyruvate and a mean relative tail current of 96.8% was recorded in the cells treated with the vehicle only. The positive control item, 100 nM E-4031, reduced the relative tail current to 6.0% of the original level.

The relative tail current amplitude of the groups treated with 8 mg/ml sodium pyruvate or with the hyperosmolar control did not differ significantly from the vehicle group results in the initial experiment. As such, there were no relevant test item-related findings in this study. However, 4 mg/ml sodium pyruvate is reported as the maximum concentration demonstrating no current inhibition of the hERG-1 channel, as exposure could not be verified for the higher concentration.

5.1.3.2 Effects on the Cardiovascular System in the Conscious Dog

5.1.3.2.1. RCC Study 855869 (GE Study Number B101019) AH111501 Dissolved in 13C-AH110896: Effects on Cardiovascular and Respiratory Parameters in the Telemetered Dog (GLP

Four beagle dogs were dosed intravenously by slow bolus injection (5-10 ml/min). All treatments were given at a dose of 2.9 ml/kg bw. The period between each treatment was at least three days. The dogs were treated with control item (0.9% saline), TRIS/EDTA vehicle control solution,

and test item containing 44.6 mg/ml [1-13C] pyruvate and 0.85 mg/ml AH111501 and filtered test item. In addition, noradrenaline (5 µg/kg bw) was used as a positive control substance to test the sensitivity of the model. Blood pressures, heart rate and electrocardiograms were monitored up to 120 min after dosing in the restrained animals.

Clinical signs were monitored throughout the experiment and at least once on return of the animals to their home cage. Respiratory rate and tidal volume were monitored. Blood was sampled to verify exposure and for pharmacokinetic analysis.

Administration of control item (0.9% saline) or vehicle control solution had no overt effects on the measured parameters. Apart from licking during application, animals remained symptom- free after treatment with control item (0.9% saline) or vehicle control solution. Intravenous injection of the test item containing AH111501 had no marked effects on the measured parameters when compared to control item (0.9% saline). Likewise, there were no noticeable effects when the test item containing AH111501 was compared to either vehicle control solution or to filtered test item. There were no changes in ECG traces indicative of any effect of either test item. Some minor clinical signs, which included licking and slight ptosis, were noted during dosing with test item. Intravenous injection of noradrenaline induced the expected effects on the cardiovascular parameters, confirming the sensitivity of the test system.

The blood samples were analyzed for [1-^{13}C]pyruvate and pyruvate and [1-^{13}C]lactate and lactate. Following administration of [1-^{13}C]pyruvate, rapid metabolism into [1-^{13}C]lactate was observed. The combined blood concentrations of [1-^{13}C]pyruvate and [1-^{13}C]lactate suggested that substantial elimination or distribution into tissues had taken place before the first blood sampling time point (5 min). [1-^{13}C]pyruvate was eliminated from the blood with an average half-life of 14±3 min, with no significant difference between the treatment groups. The average terminal elimination half-life of [1-^{13}C]lactate was 8±2 min. The presence or absence of AH111501 in the injection had no statistically significant effect on any of the examined pharmacokinetic parameters.

In conclusion, slow intravenous administration of test item containing [1-^{13}C]pyruvate and AH111501 at a dose of 2.9 ml/kg bw had no significant effects on the cardiovascular and respiratory parameters monitored in dogs. When dose levels were expressed as Human Equivalent Doses based on body surface area, they correspond to 72 mg/kg bw of [1-^{13}C]pyruvate and 1.4 mg/kg bw of AH111501.

5.1.3.2.2. Porsolt study number: 06.075/5 (GE study number B101036) Effects of rapid intravenous administration of a mixture of natural abundant pyruvic acid and 15 mM AH111501 dissolved in TRIS/EDTA dissolution medium on the cardiovascular function in the conscious telemetered dog (GLP)

The effects of 250 mM pyruvate and 0.43 mg/ml AH111501 in TRIS/EDTA dissolution medium on arterial blood pressure, heart rate and the main parameters of the electrocardiogram were evaluated following rapid intravenous injection in the conscious male beagle dog. The animals were monitored by telemetry. Each dog was administered: three doses of test item containing AH111501 (1.4, 4.3 and 5.7 ml/kg bw); three doses of filtered test item (1.4, 4.3 and 5.7 ml/kg bw); and three controls (saline, osmotic control [mannitol] and TRIS/EDTA vehicle control; all at 4.3 ml/kg bw). All doses were administered at 5 ml/s by power injector. The dogs were assigned to the

various treatments in a random manner such that each dog had each treatment once. Each animal was given a total of nine discreet IV bolus injections. There was a minimum of 48 h wash-out between each single administration. Baseline values for the measured parameters were reported before the start of injection (average of -1 and -2 min values) and post-injection values were reported at 0.5, 1, 2, 3, 5, 10, 20 and 60 min after the start of administration. Samples of the test articles were analyzed for purity by HPLC and selected samples were analyzed for purity by NMR. The pH and osmolality of all samples were measured. In addition, blood was sampled for pharmacokinetic analysis of pyruvate and lactate at 3 min before and at 1, 5, 10, 30 and 60 min after the start of each administration.

The purity profiles of the administered test articles were qualitatively consistent and appropriate for the study.

The bioanalytical data confirmed correct administration of the test item. Pyruvate was rapidly metabolized to lactate following intravenous injection, with high concentrations observed as early as 1 min post-dosing. T_{max} was in the range of 1 to 10 min. The pharmacokinetics of pyruvate and lactate were calculated using non-compartmental analysis. The blood concentrations of pyruvate and lactate were corrected for pre-dose levels of the analytes before pharmacokinetic analysis. There were no statistically significant differences in C_{max} or AUC_{tot} between filtered and unfiltered test substance in any of the dose groups, suggesting that the presence of AH111501 in the test article did not influence the blood concentration of pyruvate. Pyruvate was eliminated from blood with an average elimination half-life of 18±11 min. Dose- normalized pyruvate data indicated that C_{max} deviated negatively from proportionality in the 1.4 ml/kg bw dose group. AUC_{tot} increased proportionally with dose. Dose-normalized lactate data indicated that C_{max} and AUC_{tot} deviated positively from proportionality in the 1.4 ml/kg bw dose group. Due to the individual variation in data, the biological significance of these findings is not known. For further pharmacokinetics data see section 5.2.8 Other Pharmacokinetic Studies.

Administration of saline caused a slight and non-significant increase in blood pressure of 6% over baseline and a larger, but still not significant, increase in heart rate of 23% over baseline at 2 min after dosing. TRIS/EDTA vehicle control caused a significant increase in heart rate at 30 s when compared to baseline, but the effect was not significant when compared to saline. The osmotic control caused a decrease in heart rate at 2 min after dosing which was significantly different from saline, but not from baseline. The test item containing AH111501 and the filtered test item caused comparable effects. At doses of 4.3 and 5.7 ml/kg bw the test articles caused a non-significant, transient and slight increase in arterial blood pressure. At 5.7 ml/kg, however, the slight increase was preceded by a non-significant, weak and transient reduction in arterial blood pressure immediately after administration. The dose of 1.4 ml/kg of the filtered test article caused a slight decrease in heart rate at 2 min like the effect observed for the osmotic control. The decrease was significant compared to saline but not significant when compared to baseline. Heart rate was significantly increased compared to baseline by doses of 4.3 and 5.7 ml/kg for the test item containing AH111501 and 4.3 ml/kg of the filtered test article. The increases for the 5.7 ml/kg bw dose of test item containing AH111501 and the 4.3 ml/kg bw dose of filtered test article were also significant when compared to saline. No substantial effects on the PR- or QT-intervals were observed after administration of the pyruvate test articles. The test articles did not significantly modify the QTc interval (Fridericia's and van de Water's formulae) as compared with physiological saline. However, when compared with baseline, a significant lengthening of the QTc interval was observed when using the Fridericia's formula at 4.3 and 5.7 ml/kg of the test article containing AH111501 and at 4.3 ml/kg bw of the filtered test article. This apparent lengthening was mainly ascribed to the delay in the QT-interval adaptation to the sudden

variation of heart rate. In the presence of tachycardia without a clear reduction in the QT-interval (e.g. +47% in heart rate and -3% in QT-interval at 30 s after injection of a dose of 5.7 ml/kg bw of unfiltered test article), the formula used to correct the QT-interval does not appear to be totally efficient. In addition, this lengthening was very slight when van de Water's formula was applied. Therefore, this transient lengthening in the QTc interval (Fridericia's formula) was considered to have little, if any, biological relevance.

At a dose of 1.4 ml/kg the test articles had no biologically relevant effects in the telemetered dog. The dose of 1.4 ml/kg 250 mM pyruvate with or without 0.43 mg/ml AH111501 in TRIS/EDTA dissolution medium administered at 5 ml/s was therefore considered the No-Observed-Adverse-Effect-Level for this study.

5.1.3.3 Effects on the Cardiovascular Systems (CVS) in the Pentobarbital/Fentanyl Anesthetized Dog

5.1.3.3.1 GE study number: B101026 Cardiovascular assessment in anaesthetized dog of pyruvate administered at high injection rates (non-GLP)

Five mongrel dogs were anesthetized with pentobarbital/fentanyl and mechanically ventilated. This anesthesia offers very stable baselines for blood pressures and heart rate allowing measurement of small changes from baseline. The anesthesia causes a suppression of the baroreceptor reflexes and the dog anesthetized with pentobarbital/fentanyl is therefore considered as a sensitive model for orthostatic hypotension. The dogs were instrumented for measurement of arterial blood pressure, pulmonary arterial pressure, lead II ECG and peripheral arterial flow. Exhaled O_2 and CO_2 were recorded. A test article of 500 mM pyruvate in TRIS/EDTA dissolution medium (~990 mOsm/kg) was administered at doses of 0.71, 1.43, 2.14, and 2.86 ml/kg as rapid intravenous bolus injections at 5 ml/s. The test article did not contain any AH111501. Physiological saline, TRIS/EDTA vehicle control and an osmotically- matched glucose solution (~1030 mOsm/kg) were used as controls at a volume of 2.86 ml/kg bw. The measured responses were averaged over 15 s at 1 min before dosing and at 0.5, 1, 2, 3, 5, 10, 15 and 30 min after dosing. The data were normalized for baseline. To verify exposure and for pharmacokinetic analysis, blood was sampled at 3 min before dosing and at 1, 3, 5, 10 and 15 min after start of injection of pyruvate, and at 3 min before dosing, and at 3 and 15 min after start of injection of control solutions.

One female dog died shortly after receiving the first injection. The first dose in this individual was 2.86 ml/kg pyruvate. This animal had received approximately 4.5 times as much pentobarbital, and more than 1.6 times as much fentanyl per h when compared to the average dose given to the other four dogs. The high doses of anesthetics are thought to have abolished all cardiovascular compensatory mechanisms. Data from this animal were excluded from analysis. The remaining 4 dogs received all treatments.

Correct dosing was confirmed for all pyruvate administrations by analysis of blood samples for concentration of pyruvate and lactate. Pyruvate was rapidly metabolized to lactate, with a lactate T_{max} at approximately 5 min post-dosing.

The pharmacokinetics of pyruvate and lactate were calculated using non-compartmental analysis. The blood concentrations of pyruvate and lactate were corrected for pre-dose levels before pharmacokinetic analysis. Systemic exposure to pyruvate, measured by C_{max} and AUC_{tot} increased proportionally with dose, with the highest concentrations measured at the first sampling time

point (1 min post-dosing). The dose proportionality of lactate exposure was difficult to evaluate, as the concentrations observed in the lowest dose group were low compared to the variability in pre-dose lactate concentrations. However, both C_{max} and AUC_{tot} showed a negative deviation from proportionality at the highest dose level. For further pharmacokinetics data see section 5.2.8 Other Pharmacokinetic Studies.

The pyruvate test article was found to cause a dose-dependent acute decrease in systemic blood pressure (30 s) immediately followed by a dose-dependent compensatory increase in heart rate (1 min). The increases in heart rate were mirrored by increases in peripheral flow (1 min). Pulmonary arterial pressure also increased in a dose-dependent manner with the peak effect at 2-3 min. Whereas the effects on blood pressure and flow were rapidly reversed, the effect on heart rate persisted for up to 10 min and the increase in pulmonary arterial pressure persisted for up to 10-15 min. Whether the increase in pulmonary arterial pressure was a reflex effect caused by an increase in cardiac output or due to an increase in vascular resistance over the pulmonary system could not be judged from the measured parameters. A short-lasting dose- dependent prolongation of the QT/QTcV-interval was observed after administration of pyruvate test article. No evidence of treatment-related arrhythmias was recorded. Administration of TRIS/EDTA vehicle control caused an acute short-lasting decrease in blood pressure. Administration of osmotically-matched glucose solution caused effects like those of pyruvate. In two animals the 2.14 ml/kg dose of pyruvate dose was repeated at a reduced injection rate of 2 ml/s. The reduction in injection rate appeared to diminish the effects on systemic arterial pressure and heart rate and to a lesser degree the effect on pulmonary arterial pressure.

5.1.3.3.2 GE study number: B101031 Mechanistic study of effects of 500 mM pyruvate on the cardiovascular system in anaesthetized male dogs (non-GLP)

Hemodynamic effects of injections of a 500 mM pyruvate test article in TRIS/EDTA dissolution medium were studied in an open-chest model in three heavily instrumented, pentobarbital/fentanyl anesthetized dogs. The model allowed for continuous measurement of cardiac output. Femoral arterial flow was measured a cannula was placed downstream in the same artery for performing local injections. Systemic and pulmonary arterial blood pressures were measured. The left-ventricular end-diastolic pressure was measured as a surrogate for pulmonary venous pressure. Heart rate was generated from ECG lead II. No assessment of the ECG trace was done. A catheter was inserted in the left atrium. By administering test item directly into the left atrium, the pulmonary system was circumvented in the first pass. Doses of the test article were diluted with water for injection (WFI) to isotonicity and administered at comparable dose-rates (mg/s) to the hyperosmotic solution both systemically (intravenously) and locally (arterially). The appropriate saline volume controls were administered. Effects of the isotonic solutions were considered to reflect pyruvate effects and volume effects. A dose of 2 ml/kg bw was administered at both 2 and 5 ml/s in each dog. Some samples of the administered test articles were obtained and analyzed for purity by HPLC, pH and osmolality.

Most of the test article samples (13 of 18 samples) had a purity of more than 90% area. The lowest measured purity was 78% area. The osmolality was approximately 1000 mOsm/kg for the hypertonic test articles and 280 mOsm/kg for the isotonic test articles. pH varied from 5.6 to 8.0.

Systemic administration of the hypertonic pyruvate test article caused an acute drop in arterial blood pressure, in accordance with Study B101026. A secondary moderate increase in arterial pressure of up to 10% over baseline was observed at approximately 5 min after administration.

When the hypertonic test article was injected in an artery in the periphery an acute local increase in flow was induced indicating local vasodilation. The increase in flow was solely dependent on the osmolality of the test article, as injection of the same dose of pyruvate in an isotonic test article over the same time (i.e. at a higher injection rate [ml/s]) caused no increase in local flow when compared to a volume control. However, when comparable doses of pyruvate in hypertonic and isotonic test articles were administered intravenously at the same dose-rate, there were only minor differences in response. This finding of different effects of local versus systemic administration can possibly be explained by pyruvate being metabolized to lactate which is known to be a vasodilator. This might take place during contact with blood [Romijn et al 1994], explaining the difference between central and peripheral injection responses.

There was a consistent and acute rise in aortic flow of about 20% to 25% after intravenous injections of 2 ml/kg 500 mM pyruvate compared to an increase of approx. 16% after injection of 2 ml/kg saline. This increase indicates that no adverse effect on the heart's pumping function occurred following administration of pyruvate. The cardiac workload was calculated as the product of beat-by-beat averages of aortic flow and arterial pressure. This parameter can be considered an indicator for the relative oxygen demand of the myocardium. The typical pattern consisted of an initial reduction in cardiac work, followed by a sustained elevation of up to 30% above baseline. The dip coincided with the initial drop in blood pressure, while the increase in cardiac work occurred in the time period with moderately elevated arterial pressure and elevated aortic flow. Injection of 2 ml/kg of saline produced a sustained increase in power of some 20%, without the initial dip.

There was a close correlation between the increase in cardiac output and the observed increase in pulmonary arterial pressure, indicating that the increase in pulmonary arterial pressure was a reflex of increased flow. Small increases in pulmonary venous pressure were observed following administration of up to 2 ml/kg of 500 mM pyruvate. The increases were well within the range of normal physiological variation that occurs in response to postural changes. No evidence of increased resistance over the pulmonary circulation was observed. When bypassing the lung by injecting directly into the left atrium, no substantial difference was seen in pulmonary resistance at the relevant time interval for first-pass. The effect on the systemic blood pressure was somewhat increased, probably due to reduced mixing of the injected bolus with blood resulting in a higher concentration in first-pass. Reducing the rate of injection from 5 to 2 ml/s lessened the effect on systemic blood pressure, but had little effect on the changes seen for pulmonary arterial and venous pressures.

5.1.3.3.3 Porsolt study number: 06.076/6 (GE study number B101037) Safety pharmacology study of hemodynamic effects of a mixture of natural abundant pyruvic acid and 15 mM AH111501 dissolved in TRIS/EDTA dissolution medium after intravenous administration in the anesthetized dog (GLP)

The effects of 250 mM pyruvate and 0.43 mg/ml AH111501 in TRIS/EDTA dissolution medium on systemic, cardiac, and pulmonary hemodynamics were evaluated following rapid intravenous injection (5 ml/s) by a power injector in four anesthetized male beagle dogs. The first dose administered to all animals was a dose of filtered test item (4.3 ml/kg bw), following this dose three doses of test item containing AH111501 (1.4, 4.3 and 5.7 ml/kg bw) and three controls (saline, osmotic control [mannitol] and TRIS/EDTA vehicle control, all 4.3 ml/kg bw) were administered. Each dog received each treatment once, and between two administrations of the test item, a vehicle control was administered. Each dog was given a total of seven discrete

intravenous boluses on one dosing day, at intervals of 45 min. The dogs were instrumented for measurement of systemic and pulmonary arterial blood pressures. Continuous recording of the left ventricular pressure was used for calculation of dP/dt_{max}, and the left- ventricular end-diastolic pressure was used as a surrogate for pulmonary venous pressure.

Femoral and carotid arterial flow was measured. Cardiac output was measured by thermodilution. Heart rate was generated from ECG lead II. The measurements were reported before the start of injection (-5 min) and at 0.5, 1, 2, 5, 10, 20, 30 and 40 min after start of administration. Blood was sampled before and after the start of injection to verify correct administration. Samples of the test article were analyzed for purity by HPLC and selected samples were analyzed for purity by NMR. In addition, pH and osmolality were measured for all samples. The purity profile for the administered test articles was qualitatively consistent and appropriate for the study. Correct dosing was confirmed by blood analysis.

Administration of test item containing 0.43 mg/ml AH111501 caused an acute dose-dependent peripheral vasodilation triggering a reduction in systemic arterial blood pressure, reflex tachycardia and an increase in cardiac output. dP/dt_{max}, which is an index of myocardial contractility, was transiently decreased at the same time as the arterial blood pressure was reduced. Left cardiac work (an index of myocardial work) was unchanged for about the first minute after dosing. Left-ventricular end-diastolic pressure and pulmonary arterial blood pressure increased, with peak effects at 1 and 5 min, respectively. Systolic ejection time was increased with a peak at 2 min. After the acute phase, systemic arterial blood pressure, dP/dt_{max} and left cardiac work increased with peak effect around 5 min. Stroke volume was also increased and peaked around 10 min. This finding suggests that the observed persistent increase in cardiac output was due to an increase in myocardial contractility together with concomitant tachycardia. Cerebral blood flow increased with peak effect between 2 to 5 min, presumably because of the increased cardiac output. The PR interval was transiently shortened, at the same time as heart rate peaked. The QTc (van de Water's formula) interval was transiently lengthened after administration. Filtered test item, evaluated at 4.3 ml/kg showed effects like those observed for pyruvate containing 0.43 mg/ml AH111501 at the same dose level. However, despite the similar fall in total peripheral resistance caused by the filtered and the unfiltered test articles (-40% versus -41%, respectively), the hypotension was slightly less pronounced in the filtered test item-treated group (-13% versus -24% for filtered vs. unfiltered, respectively), presumably because of a greater reflex tachycardia (+41% versus +31% for filtered vs. unfiltered, respectively). Furthermore, it was observed that left cardiac work increased immediately after administration of filtered test item. This was contrary to the observation for test item containing AH111501 at the same dose.

The injection of 1.4 ml/kg of test item containing AH111501 caused small but significant decreases of 10% in mean aortic pressure and 7% in systolic aortic pressure at 30 s when compared to baseline. The decrease in mean aortic pressure was not significant when compared to saline control (4.3 ml/kg) whereas the decrease in systolic aortic pressure was significant. No other significant effects were observed for this dose when compared to saline control. The dose of 1.4 ml/kg 250 mM pyruvate and 0.43 mg/ml AH111501 in TRIS/EDTA dissolution medium was therefore considered the No-Observed-Adverse-Effect–Level (NOAEL) for this study.

5.1.3.3.4 Porsolt study number: 06.434/4 (GE study number: B101049) Safety pharmacology study of hemodynamic effects after intravenous administration in the anesthetized dog of a mixture of natural abundant pyruvic acid and 15 mM AH111501 dissolved in TRIS/EDTA dissolution medium with AH111501 subsequently removed by solid phase extraction (GLP)

The study was a follow-up to Porsolt Study 06.076/6. The objective of the study was to explore the dose-effect relationship for doses from 1.4 to 4.3 ml/kg bw of filtered test item on cardiovascular parameters in the pentobarbital/fentanyl anesthetized dog. Because no decrease was observed in carotid arterial flow following administration of the pyruvate test articles in Study 06.076/6, and the flow moreover was moderately increased with increases in cardiac output ensuring adequate blood supply to the brain, this parameter was not monitored in this study. All other instrumentation was kept as in Study 06.076/6. Ejection time was not measured from the aortic blood pressure trace in the present study. Left-ventricular end diastolic pressure was again used as a marker for pulmonary venous pressure. The same sampling time points were used as in Study 06.076/6. Filtered test item and saline volume controls were administered at doses of 1.4, 2.1, 2.9, 3.6 and 4.3 ml/kg at 5 ml/s with a power injector giving in total 10 discrete injections per dog. A wash-out period of 45 min was applied between all injections. Each dog received each treatment once, and the dosing sequence alternated between administrations of the filtered test item and saline. The administrations were paired so that the total volume of the filtered test item plus the following control was 5.7- 5.8 ml/kg.

Area under curve (AUC) was calculated for the duration of the peak effects of mean aortic blood pressure, left-ventricular end-diastolic pressure, pulmonary arterial pressure, heart rate, cardiac output, total peripheral resistance, stroke volume and left cardiac work. The duration of the peak effect for the different parameters was determined in advance, based on data from Study 06.076/6. AUC calculations were made for absolute change from baseline values.

The purity profile for the administered test articles was qualitatively consistent and appropriate for the study. Correct dosing was confirmed by blood analysis.

As seen in Study 06.076/6 rapid bolus injections of pyruvate test article caused acute dose-dependent peripheral vasodilation, triggering dose-dependent acute decreases in systemic arterial blood pressure and compensatory increases in heart rate. An acute and prolonged increase in cardiac output was observed. Left cardiac work increased immediately after administration. The initial increases (at 30 s) in cardiac work were like the increases observed with the respective saline controls. In Study 06.076/6 a similar pattern was seen for the filtered test article whereas the increase did not occur before 1 to 2 min after administration of the test article containing 0.43 mg/ml AH111501.

Considering the measured effects as changes from baseline, the magnitude of the peak effect compared to that of the appropriate saline control, and AUC (where available) compared to the appropriate saline control, the effect level was 2.1 ml/kg bw of pyruvate in this study. The dose of 1.4 ml/kg bw of pyruvate caused minor but statistically significant peak effects when compared to the saline volume control. It caused an acute decrease in mean aortic pressure of 8% for test item and 2% for saline (equal to -8 and -2 mmHg, respectively). $AUC_{-5\ to\ 2\ min}$ for mean aortic pressure was, however, not significantly different between the two treatments. Administration of the dose caused an acute increase in left-ventricular end diastolic pressure of 2 mmHg compared to 1 mmHg for the saline control, but this difference was not significant as

regards the $AUC_{-5\ to\ 5\ min}$. For pyruvate, the decrease in systemic blood pressure caused a decrease in dP/dt_{max} of 15% whereas saline control caused a decrease of 3%. Pyruvate caused a significant acute increase in heart rate of 17% compared to 5% for saline. This effect was also significant for $AUC_{-5\ to\ 20\ min}$. The rapid change in heart rate caused a slight increase in QTcV of 4% (equal to 13 ms) compared to 2% (equal to 6 ms) for saline. For peripheral resistance, pulmonary arterial pressure, cardiac output, left cardiac work and stroke volume as well as for the PR-and QT-intervals there were no effects when compared to the volume control. However, as the effects described above are considered well within normal physiological variation, the dose of 1.4 ml/kg bw of filtered test item was considered to be the No-Observed-Adverse-Effect–Level (NOAEL) for this study.

5.1.3.4 Effects on the Central Nervous System (CNS) and general physiology

5.1.3.4.1 RCC study 855869 (GE study number: B101011) AH111501 dissolved in 12C-AH110896: Modified Irwin screen test in the rat (GLP)

Two test items were used in this study. Test item containing 500 mM (44 mg/ml) pyruvate and 0.85 mg/ml AH111501 in TRIS/EDTA dissolution medium and filtered test item. Male Wistar rats were assessed for behavioral changes after a single administration of test item containing AH111501 at dose levels of 1, 3 or 5 ml/kg bw. The control item (0.9 % saline), TRIS/EDTA vehicle control solution and the filtered test item were only administered at doses of 5 ml/kg bw Observations included home-cage assessments as well as the response to a new environment (arena) and handling by a technician. Observations were made before dosing, shortly after dosing and at 15 min, and 1 and 2 h after administration.

A dose-dependent increase in the appearance of a blue colored tail tip was recorded in all animals treated with test item containing AH111501. This was not observed in animals that received the filtered test item. There was a significant increase in the incidence of meiosis immediately after treatment with vehicle control solution when compared to saline-treated animals. This observation was also evident in all animals treated with 5 ml/kg bw of the test item containing AH111501 or filtered test item. This effect was considered to be vehicle related.

In conclusion, intravenous administration of test item containing AH111501 and filtered test item up to doses of 5 ml/kg bw had no significant effects on the behavior of male Wistar rats when evaluated in a modified Irwin screen test. When dose levels were expressed as Human Equivalent Doses based on body surface area, this corresponds to 36 mg/kg bw of pyruvate and 0.7 mg/kg bw of AH111501.

5.1.3.4.2 Porsolt study number: 06.210/5 (GE study number: B101039) Evaluation of a mixture of natural abundant pyruvic acid and AH111501 dissolved in TRIS/EDTA dissolution medium on EEG and behavior in the conscious dog (Non-GLP)

The potential effects of 250 mM pyruvate and 0.43 mg/ml AH111501 in TRIS/EDTA dissolution medium on general behavior and EEG activity was assessed in three non-naïve conscious beagle dogs. Each dog was given one dose of the test item containing AH111501 (4.3 ml/kg bw), three doses of filtered test item (1.4, 4.3 and 5.7 ml/kg bw), three controls (saline, osmotic [mannitol] and TRIS/EDTA vehicle control, all 4.3 ml/kg bw) and on the last dosing day pentylentetrazole (PTZ, 1.5 mg/kg/min) was administered as a positive control. All injections except PTZ were

administered at 5 ml/s by power injector. The dogs were assigned to the various treatments in such a manner that each dog had each treatment once and that between two administrations of the test item, a vehicle control was administered. Each dog was given a total of eight discrete IV bolus injections. There was a minimum of 48 h wash-out between each single administration.

The dogs were implanted with EEG electrodes in the cortex and the signal was monitored by telemetry. For 30 min before and for a minimum of 20 min after each administration the dogs were restrained in a sling. EEG traces were recorded from 20 min before dosing and for 3 h after. The traces were assessed by visual inspection of the raw EEG signals. The general physiological state of the animals was scored on 13 parameters (respiratory rate, cutaneous pinch reflex, agitation and/or vocalization, salivation, etc.) at 5 min before dosing and at 5 and 20 min after dosing. Blood was sampled before and after administration to confirm correct dosing. Samples of the test articles were analyzed for concentration and purity of pyruvate by HPLC and selected samples were analyzed for purity by NMR. pH and osmolality were measured for all samples.

The purity profiles of the administered test item test articles were qualitatively consistent and appropriate for the study. Some of the samples were found to contain low levels of ethanol, probably caused by conditioning of the chromatography column (for extraction of AH111501) with 96% ethanol, followed by insufficient flushing with TRIS/EDTA vehicle control solution. One saline control was found to have unexpectedly low osmolality. When the study documentation was checked it was confirmed that a dose of water for Injection had been administered in error. Correct administration of test item was confirmed for all but one injection (in the 1.4 ml/kg bw dose group) where the post-dosing pyruvate concentration in blood neither confirmed nor disproved correct dosing.

Rapid bolus injections of the test item did not provoke pathological clinical symptoms or pathological EEG activity. The occasional symptoms observed were of a non-specific nature, transient or appeared before and after substance administrations and could not be attributed to either the substance or the composition of the vehicle. Convulsions and other pathological signs such as those reported in the mouse after IV injections of 10 ml/kg of 0.9 M and 1.8 M sodium pyruvate injections [Gonzalez et al. 2005] were not observed in any of the 3 dogs tested. However, convulsions were observed following PTZ infusion in all 3 dogs, confirming the sensitivity of the model.

In conclusion, intravenous administration of test item containing 250 mM pyruvate and 0.43 mg/ml AH111501 at a dose of 4.3 ml/kg and filtered test item up to doses of 5.7 ml/kg bw administered at 5 ml/s had no significant effects on the general behavior and EEG activity of male beagle dogs. When maximum dose levels were expressed as Human Equivalent Doses based on body surface area, this corresponds to 70 mg/kg bw of pyruvate and 1.0 mg/kg bw of AH111501.

5.1.3.5 Effects on the Respiratory System in the Conscious Dog

5.1.3.5.1 RCC study 855869 (GE study number B101019): AH111501 dissolved in 13C-AH110896: Effects on cardiovascular and respiratory parameters in the telemetered dog (GLP)

Four beagle dogs were dosed intravenously by slow bolus injection (5-10 ml/min). All treatments were given at a dose of 2.9 ml/kg bw. The period between each treatment was at least three days. The dogs were treated with control item (0.9% saline), TRIS/EDTA vehicle control solution, and test item containing 44.6 mg/ml [1-13C] pyruvate and 0.85 mg/ml AH111501 and filtered test item. Animals were trained several times to accustom them to dosing procedures, as well as respiratory parameters measurements. Tracheal airflow was measured using a sealed mask

(covering the animal's snout) attached to a pneumotachograph and a pulmonary monitoring system. Respiratory rate and tidal volume were monitored in the sling-restrained animals. Values were recorded for at least approx. 4 to 5 min before administration and at 15, 32, 62 and 122 min after the end of dosing. Mean values over 3 min were reported.

The various treatments had no marked effects on absolute and percent change values of respiratory parameters (respiratory rate, tidal volume and minute volume) when measured at approximately 15, 32, 62 and 122 min after treatment. There were no significant differences between the treatment groups.

In conclusion, slow intravenous administration of test item containing [1-^{13}C]pyruvate and AH111501 at a dose of 2.9 ml/kg bw had no significant effects on the respiratory parameters monitored in dogs. When dose levels were expressed as Human Equivalent Doses based on body surface area, they correspond to 72 mg/kg bw of [1-^{13}C]pyruvate and 1.4 mg/kg bw of AH111501.

5.1.3.6 Discussion and Conclusions for Safety Pharmacology

Test articles of 500 mM [1-^{13}C]pyruvate or pyruvate with 0.85 mg/ml AH111501 dissolved in TRIS/EDTA dissolution medium were used in the initial safety pharmacology studies of effects on the cardiovascular and respiratory systems (telemetered dog) and the central nervous system (Irwin rat). There were findings of slight ptosis (dog) and vehicle-related meiosis (rat) in these studies, but no other adverse reactions were recorded. The injection volumes and rates used in these studies were in line with current good practice [Diehl et al. 2001]. Because the intended clinical injection rate is 5 ml/s, a non-GLP study of effects on the cardiovascular system (CVS) was conducted in the pentobarbital/fentanyl anesthetized dog using the clinical injection rate and a test article of 500 mM pyruvate in TRIS/EDTA dissolution medium. This test article did not contain AH111501. The anesthesia offers very stable baselines for blood pressures and heart rate allowing measurement of small changes from baseline. The anesthesia also causes a suppression of the baroreceptor reflexes and the dog anesthetized with pentobarbital/fentanyl is therefore to be considered a sensitive model for orthostatic hypotension. Finally, the anesthesia causes a reduction in cardiac output compared to a conscious animal thereby resulting in slower dilution of the injected bolus and higher blood concentration in the first pass. The study demonstrated that when administered as a rapid intravenous bolus the 500 mM pyruvate test article caused an acute decrease in arterial blood pressure which was momentarily followed by a compensatory increase in heart rate and an increase in peripheral flow.

Administration of an osmotically matched glucose solution caused a similar effect. The decrease in blood pressure was explored in a mechanistic study (non-GLP) where hypertonic and isotonic pyruvate test articles without AH111501 were injected. In this study it was demonstrated that the decrease in systemic blood pressure was caused by peripheral vasodilation caused in part by the osmolality of the test article and in part by pyruvate itself or by a metabolite of pyruvate (possibly lactate). The effects of rapid boluses (5 ml/s) of a diluted test article containing 250 mM pyruvate and 0.43 mg/ml AH111501 dissolved in TRIS/EDTA dissolution medium were tested in two studies in the conscious telemetered dog (CNS [non-GLP] and CVS) and in two studies in the anesthetized dog (CVS). When administering the same dose of pyruvate (mg/kg) at 5 ml/s, the reduction in osmolality of the test article, possibly in combination with the lower dose-rate (mg/s), gave the anticipated reduction in the observed effects on blood pressure and heart rate. The minor effect on QTcV observed in the anesthetized dog after administration of 1.4 ml/kg bw filtered test article (Porsolt 06.434/4) was considered an effect of the rapid change in heart rate rather than an effect on hERG ion-channel. This is supported by the lack of effect of sodium

pyruvate and AH111501 in the hERG assay.

The No-Observed-Adverse-Effect-Level (NOAEL) in these studies was 1.4 ml/kg bw for the cardiovascular studies and 5.7 ml/kg for the CNS study. In summary, the available nonclinical safety pharmacology data support adequate safety of Hyperpolarized Pyruvate (^{13}C) Injection containing 250 mM [1-^{13}C]pyruvate and no-more-than 3 µM of AH111501 for use in human prostate cancer patients. The data from the nonclinical studies in conscious and anesthetized dogs, however, also indicate that in special patient subpopulations (e.g. patients treated with high doses of adrenoceptor antagonists, and patients suffering from severe heart failure rapid administration of this test article may cause cardiovascular effects if administered in large volumes (>1.4 ml/kg bw). In the planned Phase 1/2a clinical imaging study the maximum dose given to any subject in an ascending dose design will be 0.43 ml/kg bw of Hyperpolarized Pyruvate (^{13}C) Injection. The safety of this dose has previously been demonstrated in clinical studies in healthy adult (18 to 45 years of age) volunteers and in healthy elderly (age ≥ 60 years) volunteers.

5.1.4 Pharmacodynamic Drug Interactions

No studies have been performed to date.

5.2 Pharmacokinetics

5.2.1 Brief Summary

Biodistribution studies with [1-^{14}C]pyruvate in rats showed rapid distribution of radioactivity throughout the body following intravenous injection. The radioactivity concentration in blood corresponded to a total blood recovery of 8.9% ±1.3% of injected dose (id) at 30 s post-dosing. Hence, more than 90% of the injected radioactivity dose was distributed out of the blood at 30 s post-dosing. The initial volume of distribution corresponded approximately to that of total body water.

The highest concentration of radioactivity 30 s post-dosing was found in the pancreas. This may reflect formation of [1-^{14}C]oxaloacetate through pyruvate carboxylase in pancreatic islets. Relatively high concentrations were also found in highly perfused tissues such as the liver, adrenal glands, heart muscle and the small and large intestine walls.

The lowest concentrations of radioactivity were found in white fat, testes, brain and spinal cord. The highest total recovery was found in skeletal muscle and skin.

The major elimination route was through exhaled air. At 24 h post-dosing, radioactivity corresponding to 56% of id was recovered in exhaled air, because of the formation of $^{14}CO_2$. Only minor amounts of radioactivity were excreted in urine and feces (2.1% and 0.26% of id respectively, at 24 h post-dosing). The recovery from exhaled air suggest that the majority of the injected [1-^{14}C]pyruvate enters the citric acid cycle through formation of acetyl-CoA.

Blood samples collected following intravenous injection of pyruvate in dogs demonstrated rapid metabolism of pyruvate to lactate. The systemic exposure, assessed by C_{max} and AUC, suggested

that the exposure to both pyruvate and lactate was proportional to dose. There appeared to be lower peak concentrations and more rapid conversion of pyruvate to lactate in conscious dogs than in anesthetized dogs.

The presence of AH111501 in the test item did not alter the pharmacokinetics of pyruvate.

The general impression from the available pharmacokinetic data is that the rapid distribution and conversion of pyruvate to lactate, and the subsequent entry into metabolic pathways, is entirely compatible with the intended use of the product.

5.2.2 Methods of Analysis

5.2.2.1 M101001: Quantitation of pyruvate and lactate in dog blood by liquid chromatography - mass spectrometry

A liquid chromatography-mass spectrometry (LC-MS) method used for quantitation of [1–^{13}C]pyruvate and [1-^{13}C]lactate in dog blood samples was successfully developed and validated (M101001 and V101001, respectively). The method has been used for analysis of samples collected in toxicology and safety pharmacology studies.

Blood samples were collected using vacuum tubes containing dry heparin, and 200 µl blood immediately transferred to weighed centrifuge tubes containing 900 µl methanol and 20 µl internal standards ([$^{13}C_3$]pyruvate and [$^{13}C_3$]lactate). After mixing by inversion and weighing, the tubes were kept on ice until centrifugation (less than 30 min after collection of blood) at 15000g at 4°C for 15 min. The supernatant was then transferred to a new sample vial and stored below -15°C until analysis.

The calibration range of the assay was approximately 4.5 to 4500 µM for pyruvate and 9 to 9000 µM for lactate, and the calibration curves were fitted to a non-linear equation: $y=a+bx+cx^2$ and weighted with a weighting factor of $1/y^2$. A good fit of the calibration points to the calibration curves was obtained. The concentrations of pyruvate and lactate in the standard with lowest amount of pyruvate and lactate (CS1) were defined as the analytical lower limits of quantitation (LLOQ).

The total precision of the method (RSD_t) was 9.1%, 3.1% and 8.3% for pyruvate and 5.7%, 2.4% and 3.3% for lactate, for the low, medium and high concentration quality control (QC) samples, respectively, which was within the acceptance criteria (15% RSD). The accuracy of the method (RE) was -1.9%, 2.3% and -2.4% for pyruvate and 2.0%, 6.2% and 4.1% for lactate, for the low, medium and high concentration QC samples, respectively, which was within the acceptance criteria (15% RE).

To determine recovery of the method three concentration levels of standard were diluted in whole blood from dog and in protein-free supernatant of methanol treated whole blood from dog. The recovery in whole blood compared to protein-free supernatant was 93%, 115% and 110% for pyruvate and 70%, 111% and 104% for lactate, for the low, medium and high concentration recovery blood (RB) samples, respectively. The precision at each concentration level of the recovery blood samples was within the acceptable RSD, except for the low lactate recovery sample, which is discussed in the validation report (V101001).

Furthermore, pyruvate and lactate were considered adequately stable in protein-free supernatant during 3 freeze/thaw cycles, when stored in an autosampler for 30-40 h and when stored for up to seven weeks below -15°C. Pyruvate and lactate were also stable in protein-free supernatant after derivatization, when stored at 2-8°C for 7 days.

After an overall evaluation of the results, it was concluded that the method was suitable for analysis of both pyruvate and [1-^{13}C]pyruvate, and both lactate and [1-^{13}C]lactate in dog blood samples following intravenous administration of pyruvate or [1-^{13}C]pyruvate.

5.2.2.2 M101003: Quantitation of pyruvate in buffer solution by HPLC and UV detection

A method for quantitation of pyruvate in HEPES buffer using high performance liquid chromatography (HPLC) and ultraviolet (UV) detection was developed. The method was used for analysis of samples collected as part of the hERG assay.

Representative samples of the test article of sodium pyruvate collected: A) immediately after dose test article in HEPES buffer; B) from the perfusion bath before wash-in (to detect carry over); C) from the perfusion bath at the end of the experiment (to allow determination of the exposure level); and D) at the end of the experimental day were analyzed. All samples were refrigerated (3-9°C) until analyzed.

The calibration standards (CS) were made from sodium pyruvate diluted in a buffer solution in the range 0.5 to 10 mg/ml. A calibration curve was made by plotting the integrated peak area of pyruvate against the nominal concentration of sodium pyruvate. The calibration curve was fitted to a non-linear equation: $y=a+bx+cx^2$ and weighted with a weighting factor of $1/y^2$. The concentration of pyruvate in the samples was determined by integrating the pyruvate peak for the samples and correlating this peak area to amount pyruvate using the calibration curve.

At least four of the five calibration standards had to be within ±15% of their respective nominal value. A calibration standard that failed the acceptance criteria could be discarded provided it did not change the calibration model. If the calibration standards failed to meet the requirements, the results from the sequence were rejected.

QC samples (at least in duplicate) at two concentrations were incorporated in each sequence of analysis. At least 67% of the QC samples had to be within ±15% of their respective nominal value. If the QC samples failed to meet the requirements, the results from the sequence were rejected.

5.2.3 Absorption

No absorption studies have been performed due to the intended route of administration (intravenous bolus injection).

5.2.4 Distribution

5.2.4.1 Quantitative whole-body autoradiography (QWBA)

The distribution and elimination of [1-^{13}C]pyruvate spiked with a small amount of sodium [1-^{14}C]pyruvate following intravenous bolus administration have been studied by quantitative whole-body autoradiography (QWBA) in male Sprague-Dawley rats. Three animals per time point

were sacrificed at 10 different time points from 30 s to 24 h after dosing. The animals received a total pyruvate dose of 56 mg/kg bw, of which 0.2 to 0.3 mg was sodium [1-^{14}C]pyruvate.

The disposition of radioactivity in organs, tissues and excreta following a single intravenous bolus injection of radiolabeled pyruvate in rats has been studied in two separate studies.

As QWBA measures the total radioactivity in organs and tissues, radioactivity measurements represent the combined radioactivity from pyruvate and all its radiolabeled metabolites. The expected major metabolites of [1-^{14}C]pyruvate are [1-^{14}C]lactate and [1-^{14}C]alanine, as pyruvate enters the normal metabolic pathways. In addition, pyruvate is a substrate for the pyruvate dehydrogenase complex, which converts [1-^{14}C]pyruvate to $^{14}CO_2$ and Acetyl-CoA, and for pyruvate carboxylase, which forms [1-^{14}C]oxaloacetate in an anaplerotic reaction. In the latter case, a variety of ^{14}C-labeled metabolites are formed as the radiolabel enters the citric acid cycle. Thus, the data from the QWBA studies represent the total distribution of pyruvate and several metabolites, where the aggregate contribution of the metabolites to the measured radioactivity increases with time.

5.2.4.1.1 GE study number: B101003 Study of Distribution and Excretion of [1-14C] Pyruvate in Male Sprague- Dawley Rats (non-GLP)

In this initial study, the rats had an average body weight of 225 g and received an average dose of 56 mg/kg bw of sodium pyruvate, of which 0.3 mg was sodium [1-^{14}C]pyruvate and the remainder sodium [1-^{13}C]pyruvate. The test item was administered as an intravenous bolus injection. The dosing volume was 1 ml/kg bw. The rats received an average radioactivity dose of 679 kBq per animal. The rats were anaesthetized during the study.

Three rats per time point were sacrificed at 30 s, and 1, 2, 5 and 10 min post-injection, and one rat per time point was sacrificed at 15, 30, 60 and 120 min post-injection. Exhaled air was collected from all rats sacrificed 10 min post-injection or later, and urine was collected from all rats sacrificed 15 min post-injection or later. No feces samples were excreted from any of the animals.

After IV injection, radioactivity was rapidly distributed throughout the body. A dose of 56 mg pyruvate/kg (including both [1-^{13}C] and [1-^{14}C]-labeled pyruvate) resulted in a blood concentration of radioactivity corresponding to 0.7% of id/g (equivalent to 85.5 µg pyruvate/g blood) at 30 s post-dosing (corresponding to a total blood recovery of 8.9±1.3 % of id). The initial volume of distribution was estimated to 677 ml/kg, indicating rapid distribution of radioactivity to a volume comparable to that of total body water.

The highest concentration of radioactivity 30 s post-dosing was found in the pancreas. This may reflect formation of [1-^{14}C]oxaloacetate through pyruvate carboxylase in pancreatic islets. Relatively high concentrations were also found in highly perfused tissues such as the liver, adrenal glands, heart muscle and the small and large intestine walls.

The lowest concentrations of radioactivity were found in white fat, testes, brain and spinal cord.

The concentration of radioactivity in the prostate at 30 s post-dosing was 1.87 kBq/g. This corresponds to a pyruvate concentration of 34.5 µg/g. The elimination rate in prostate during the first 2 min post-dosing corresponded to a half-life of 1.5 min.

The highest total recovery of radioactivity was found in muscle and skin tissue. At 1 min post dosing, the recovery in muscle and skin was 55% and 19% of id, respectively. At 60min post- dosing, the recovery in muscle and skin was 13% and 7% of id, respectively.

The recovery of radioactivity in whole-body cryosections decreased rapidly, from approximately 100% of id at 2 min post-injection to 67% at 10 min post-injection.

The autoradiograms indicate some elimination of radioactivity through the kidneys, but no measurable amounts of urine were produced during the time-frame of the study.

The major elimination route of radioactivity was through exhaled air. At 120 min post-dosing, radioactivity corresponding to 63% of id was recovered in exhaled air, because of the formation of $^{14}CO_2$. This suggests that in the rat a major part of the injected pyruvate rapidly enters the citric acid cycle via the Acetyl CoA pathway.

5.2.4.1.2 GE study number: B101018: Study of Distribution and Excretion of [1-14C] AH110896 in Male Sprague- Dawley Rats - follow-up of study B101003 (non-GLP)

This follow-up study was designed to determine the amount of radioactivity in organs, tissues and excreta following a single intravenous bolus injection of [1-^{14}C]-labeled pyruvate in rats, at time points from 15 min to 24 h post-dosing.

The rats used in the study had an average body weight of 176 g and received an average dose of 56 mg/kg bw of sodium pyruvate, of which 0.2 mg (for time points 2 and 24 h) or 0.3 mg (for time points 15 and 30 min and 6 h) was sodium [1-^{14}C]pyruvate and the remainder sodium [1-^{13}C]pyruvate. The rats received an average radioactivity dose of 446 kBq/animal in a dosing volume of 1 ml/kg bw administered as an intravenous bolus injection. The rats were not anesthetized during the study.

Three rats per time point were sacrificed at 15 min, 30 min, 2 h, 6 h and 24 h post-injection. Exhaled air and feces were collected from the 24-h sacrifice group, and urine and cage wash water were collected from all rats.

At 15 min post-dosing, the highest radioactivity concentrations were measured in urinary bladder contents, bone tissue and pancreas, whilst relatively high concentrations were found in liver, small and large intestine wall, bone marrow and salivary glands. The lowest radioactivity concentrations were found in brain, spinal cord, white fat and testes.

The radioactivity concentrations decreased with time in all organs and tissues. At 24 h post dosing, the highest concentrations were found in urinary bladder contents, bone marrow, large intestine wall and skin. The lowest concentrations were found in brain, white fat and eye.

The highest total amounts of radioactivity were recovered in muscle, skin and bone tissue. At both 15 min and 24 h post dosing, these tissues accounted for approximately 70% of the activity retained in the whole body.

The total recovery determined in whole-body sections decreased from 55% id at 15 min to 7.9% id at 24 h.

The major elimination route was through exhaled air. At 24 h post-dosing, radioactivity corresponding to 56% of the total id was recovered in exhaled air, because of the formation of

$^{14}CO_2$. The elimination rate decreased biexponentially with time.

Only minor amounts of radioactivity were excreted in urine and feces (2.1% and 0.26% of id respectively, at 24 h post-dosing).

The data from this study were in good accordance with data from the first study, allowing these data sets to be pooled for evaluation of the biodistribution of radioactivity in the rat following injection of sodium [1-^{14}C]pyruvate diluted with sodium [1-^{13}C]pyruvate from 30 s to 24 h after administration.

The total recovery was in the range of 53 to 67% of id at 24 h post-dosing. The total recovery is lower than expected, but since the overall results of these 2 studies indicate that in the rat IV injected pyruvate is rapidly removed from the blood and is metabolized through the expected metabolic pathways, this is still considered to be acceptable.

5.2.5 Metabolism (interspecies comparison)

As the metabolic pathways of pyruvate are well described in the literature, no specific metabolism or plasma protein-binding studies have been performed. The conversion of pyruvate to lactate was studied using bioanalytical methods both in the conscious and the anaesthetized dog, see section 5.1.3 Safety Pharmacology. The conversion of [1-^{13}C]pyruvate to [1-^{13}C]lactate was studied in the dog using magnetic resonance spectroscopy (MRS), see Study B101059 (the section on Primary Pharmacodynamics).

5.2.6 Excretion

No specific studies of excretion have been performed; reference is made to the biodistribution studies described above.

5.2.7 Pharmacokinetic Drug Interactions

No specific studies have been performed to date.

5.2.8 Other Pharmacokinetic Studies

In two safety pharmacology studies (B101026 and B101036), blood concentrations of pyruvate and lactate were studied following rapid bolus injection of mixture of pyruvic acid and AH111501 sodium salt dissolved in TRIS/EDTA dissolution medium. For further reference to these studies, see the section on Safety Pharmacology.

5.2.8.1 GE study number: B101026. Cardiovascular assessment in anaesthetized dog of pyruvate administered at high injection rates (non-GLP)

Blood samples for determination of pyruvate and lactate concentrations in blood after intravenous administration of pyruvic acid dissolved in 200 mM TRIS/EDTA dissolution medium in anesthetized dogs were collected from a femoral vein at 3 min pre-dosing, and at 1, 3, 5, 10 and 15 min post-dosing.

The pyruvate concentration was 500 mM and the dose levels were 0.71, 1.43, 2.14 and 2.86 ml/kg bw, and the dose rate 5 ml/s or 2 ml/s. The minimum interval between dosing was 35 min. For each dog a different dosing sequence was used.

Blood samples were also collected following administration of control items, for assessment of baseline pyruvate and lactate values throughout the study period.

The blood samples were analyzed using a validated reversed phase liquid chromatography mass spectrometry (LC-MS) for quantitative determination of pyruvate and lactate concentrations in whole blood.

The pharmacokinetics of pyruvate and lactate were assessed using non-compartmental analysis. The data were corrected for pre-dose concentrations prior to pharmacokinetic analysis.

The C_{max} ranged from 227±84 µg/ml in the 0.71 ml/kg dose group to 695±197 µg/ml in the 2.86 ml/kg dose group. In comparison, the blood concentration of pyruvate measured prior to the first injection on each study day was in the range from 6 to 9 µg/ml blood. The average concentration measured at 1 min post-dosing corresponded to 0.023% id/ml blood. Assuming a total blood volume of 2.0 l (9% of total body weight [Davies and Morris 1993]), the average in blood recovery, across all dose groups was estimated at approximately 50% of the id at 1 min post-dosing.

Pyruvate was rapidly eliminated from the blood. The mean elimination half-life of pyruvate was 15 min or less at all dose levels.

The bioanalytical data show that pyruvate is rapidly metabolized to lactate following intravenous injection. The lactate T_{max} was observed between 3 and 15 min post-dosing. The lactate C_{max} was approximately 2- to 3-fold lower than that of pyruvate.

The terminal elimination half-life of lactate was difficult to determine due to increase in lactate pre-dose concentrations throughout the injection sequence in each animal, probably due to incomplete elimination of the previous dose before a new dose was administered.

Systemic exposure to pyruvate increased proportionally with dose, with the highest concentrations measured at the first sampling time point (1 min post-dosing).

The dose proportionality of lactate exposure was difficult to evaluate, both due to build-up of pyruvate throughout the injection sequence, and as the concentrations observed in the lowest dose group were low compared to the variance in pre-dose lactate concentrations. However, both C_{max} and AUC_{tot} showed a negative deviation from proportionality at the highest dose level.

5.2.8.2 Porsolt study number: 06.075/5 (GE study number B101036): Effects of rapid intravenous administration of a mixture of natural abundant pyruvic acid and 15 mM AH-111501 dissolved in TRIS/EDTA dissolution medium on the cardiovascular function in the conscious telemetered dog. (GLP)

Blood samples were collected from four conscious dogs after injection of 250 mM pyruvate and 0.43 mg/ml AH111501 in TRIS/EDTA dissolution medium and after injection of filtered test item and various control solutions. The dose levels for test item containing AH111501 and filtered test item were 1.4, 4.3 and 5.7 ml/kg bw, and the injection rate 5 ml/s. Each dog received nine discrete

injections, with an interval of at least 48 h between each administration. Blood samples were collected from a jugular vein at 3 min pre-dosing, and at 1, 5, 10, 30 and 60 min after the start of each administration. The blood samples were analyzed using a validated LC-MS method (M101001).

The bioanalytical data show that pyruvate is rapidly metabolized to lactate following intravenous injection. The lactate T_{max} was observed between 1 and 10 min post-dosing.

There were no statistically significant differences in pyruvate or lactate C_{max} or AUC_{tot} between filtered and unfiltered test substance in any of the dosing groups, suggesting that AH111501 did not have any effect on pyruvate pharmacokinetics.

Pyruvate was eliminated from blood with an average terminal elimination half-life of 18±11 min. The average concentration of pyruvate in blood across all dose groups at 1 min post-dosing corresponded to 0.011±0.004% id/ml blood. Assuming a total blood volume of 1.1 l (9% of total body weight [Davies and Morris 1993], this corresponds to a recovery of 12% of the id in blood 1 min post-dosing.

Dose-normalized pyruvate data indicated that AUC_{tot} increased proportionally with dose whereas C_{max} tended to deviate negatively from proportionality in the 1.4 ml/kg bw dose group. The lower C_{max} in the lowest dose group may be attributed to the shorter injection duration in this group.

Dose-normalized lactate C_{max} and AUC_{tot} data indicated a positive deviation from proportionality in the 1.4 ml/kg bw dose group. The biological significance of this is not known.

5.2.9 Discussion and Conclusions for Other Pharmacokinetic Studies

There were some significant differences in study design between the two pharmacokinetic studies, both with respect to the test system and the test item.

The animals were either anesthetized (B101026) or conscious (B101036), and the average body weight of the dogs was nearly twice as high in the anesthetized dog study (22.8 kg) as in the conscious dog study (12.1 kg). In B101026, the dosing interval varied from 35 to 50 min, with 5 injections on one study day, whereas the dosing interval in B101036 was at least 48 h. The injection rate was identical.

The test item was formulated as a 500 mM pyruvate solution in the anesthetized dog study and a 250 mM pyruvate solution in the conscious dog study solution. Thus, with regard to the administered pyruvate dose, the 1.4 ml/kg and 5.7 ml/kg dose groups in the conscious dog study corresponded to the 0.71 ml/kg and 2.86 ml/kg dose groups in the anesthetized dog study, respectively. Throughout this section, these dose groups are referred to as the low-dose group and high-dose group. The injection rate in terms of volume/time was 5 ml/s in both studies. Therefore, the dose-rate in terms of mg pyruvate/s was higher in the anesthetized dog study.

The systemic exposure to pyruvate and lactate was assessed by C_{max} and AUC_{tot}. The general impression across both studies was systemic exposure proportional to dose for both pyruvate and lactate.

The most significant differences between the two studies were the recovery of pyruvate in blood at 1 min post-dosing, and the more rapid formation of lactate in the study in the conscious dog. At

1 min post-dosing, the average recovery in blood was estimated at 50% of id in the anesthetized dog study and as approx. 12% of id in the conscious dog study. The rapid distribution from blood is consistent with imaging data from dogs, where the C_{max} of both pyruvate and lactate in prostate is observed less than 1 min after end of injection.

It is not known whether these differences can be attributed to the differences in test item, the difference in dose rate or differences between the anesthetized and the conscious dog. It is plausible that the higher cardiac output in the conscious dog results in a more rapid distribution of pyruvate from the blood to peripheral compartments. It is also expected that a conscious animal has a higher metabolic rate compared to an anaesthetized animal.

The dose-normalized pyruvate AUC_{tot} was of the same order of magnitude in both studies.

Rapid conversion of pyruvate to lactate was observed in both studies. The formation of lactate appeared to be somewhat faster in conscious dogs than in anesthetized dogs, as the concentration measured 1 min post-dosing in the conscious dog was as high as that in the 5 min sample from the anesthetized dog. In the anesthetized dog, only minor amounts were observed 1 min post-dosing, and a significant build-up was not observed until 3 or 5 min post-dosing.

5.3 Toxicology

5.3.1 Brief Summary

Expanded single-dose GLP compliant toxicity studies have been conducted in the rat and the dog with an intravenously administered test article of [1-^{13}C]pyruvate (AH110896), containing the novel excipient AH111501. To obtain maximum exposure, the animals were dosed twice on the same day, with the maximum recommended dose volume of 20 ml/kg in the rat, and 5 ml/kg in the dog. Exposure was confirmed in the dog by analysis of blood samples. In addition to the pyruvate test article, the potential impurities, AH112623 (parapyruvate), AH112615 (reaction-product between pyruvic acid and TRIS), and AH113462 (lactone) were tested in separate, expanded single-dose GLP toxicity studies in the rat.

The pyruvate test article has also been evaluated in two *in vitro* genetic toxicology systems, comprising the bacterial reverse mutation test (Ames test) and the chromosomal aberration assay with mouse lymphoma cells (MLA test), as well as in two separate mammalian *in vivo* assays in rats, comprising the test for induction of micronuclei in the polychromatic erythrocyte of the bone marrow, and detection of DNA damage in the blood using the Comet assay. In addition, the excipient AH111501, and the potential impurities AH112623, AH112615 and AH113462 were also evaluated in the Ames and MLA tests. The potential impurity AH113462 was further tested in the *in vivo* assay for induction of micronuclei in the polychromatic erythrocyte of the bone marrow in rat.

Local tolerance studies were conducted in rabbits to investigate whether the pyruvate test article induced signs of irritation when administered by the dermal, ocular, intra-arterial, intramuscular, and subcutaneous or paravenous routes.

5.3.2 Expanded Acute-Dose Toxicity

5.3.2.1 500 mM (44.6 mg/ml) [1-13C]pyruvate and 0.85 mg/ml AH111501

5.3.2.1.1 GE study number: B101020: An expanded acute dose toxicity study with intravenously injected AH111501 dissolved in 13C Na-AH110896 in male and female rats (GLP)

Four groups of 12 male and 12 female Sprague-Dawley rats were administered either saline control, TRIS/EDTA vehicle control, low dose (892 mg [1-^{13}C]pyruvate /kg bw and 17 mg AH111501/kg bw) or high dose of the test item (1784 mg [1-^{13}C]pyruvate /kg bw and 34 mg AH111501/kg bw) of the test item. To study acute effects, an interim kill was performed on half of the animals in each group 24 h after dosing. Fourteen days post- treatment, the remaining animals were killed and necropsied.

There were no mortalities and no adverse treatment-related clinical signs. No treatment-related effects on gross pathology, body weights or organ weights were observed. No treatment- related histomorphological findings were observed. Microscopic examination of the injection site indicated that local tolerance of the test item did not differ from that of physiological saline. Changes in clinical pathology parameters were observed for the interim and the terminal groups, but these effects were not considered to be of toxicological significance and most of the changes were reversible.

The No-Observed-Adverse-Effect-Level (NOAEL) in this study was 1784 mg/kg bw for [1-^{13}C]pyruvate and 34 mg/kg bw for AH111501. The NOAEL expressed as Human Equivalent Doses (HED) based on body surface area was 288 mg/kg bw for [1-^{13}C]pyruvate and 5.5 mg/kg bw for AH111501.

5.3.2.1.2 TNO study number: 5887 (GE study number: B101017): Expanded Acute Dose Intravenous Toxicity Study with AH111501 Dissolved in 13C-AH110896 in Beagle Dogs (GLP)

Groups of three male and three female beagle dogs were treated with either saline control or with a high dose of test item (446 mg [1-^{13}C]pyruvate /kg bw and 17 mg AH111501/kg bw). Blood samples for hematology and clinical chemistry were taken 24 h and 14 days after dosing. Blood samples for toxicokinetics were collected 1 day before dosing (pre-dose) and 1, 3, 5 and 15 min post dosing. The dogs were killed 14 days after dosing for a complete post-mortem.

No test item related changes were seen in clinical signs (other than that the urine transiently had a greenish color), ophthalmoscopy, body weight, food intake, hematology, clinical chemistry, organ weights, gross findings at necropsy or in histopathology of the organs and tissues sampled.

The toxicokinetics of [1-^{13}C]pyruvate and [1-^{13}C]lactate were assessed using non compartmental analysis. The toxicokinetic endpoints were estimated individually using nominal dose volumes and exact sampling time points and summarized as mean values within each dosing group. Following intravenous administration of [1-^{13}C]pyruvate, rapid conversion to [1-^{13}C]lactate was observed. [1-^{13}C]pyruvate was eliminated from the blood with an average terminal elimination half-life of 6.4±0.9 min, with no significant difference between male and female animals. The average terminal elimination half-life of [1-^{13}C]lactate was 6.4±0.6 min. The maximum blood concentration (C_{max}) for [1-^{13}C]pyruvate was observed at the first blood sampling time point (1 min post-dosing). The C_{max} in female dogs was in the range of 2069 to 3875 µM, whereas the C_{max} in male dogs was in the range of 2066 to 2789 µM. The average C_{max} was 2700±1416 µM. The blood concentration/time profiles of [1-^{13}C]lactate suggested a concentration peak at around 3 min, with no statistically significant difference in C_{max} between male and female animals.

No-Observed-Adverse-Effect-Level (NOAEL) in this study was 446 mg/kg bw for [1-^{13}C]pyruvate and 8.5 mg/kg bw for AH111501. The NOAEL expressed as Human Equivalent Doses (HED) based on body surface area was 247 mg/kg bw for [1-^{13}C]pyruvate and 4.7 mg/kg bw for AH111501.

5.3.2.2 250 mM (22.3 mg/ml) [1-13C]pyruvate and 0.43 mg/ml AH111501

5.3.2.2.1 GE study number: B101040. An expanded acute dose toxicity study with intravenously injected AH111501 in 250 mM AH110896 dissolved in 100 mM TRIS/EDTA in male and female rats (GLP)

Six groups of 12 male and 12 female Sprague-Dawley rats were administered saline control, or TRIS/EDTA vehicle control or a high dose of the test item (892 mg [1-^{13}C]pyruvate/kg bw and 17 mg AH111501/kg bw). One group of six male and six female Sprague-Dawley rats was administered the low dose of the test item (446 mg [1-^{13}C]pyruvate/kg bw and 17 mg AH111501/kg bw). To study acute effects, an interim kill was performed in half of the saline control, vehicle-control and high-dose animals 24 h after dosing. Fourteen days post- treatment, the remaining animals were killed and necropsied.

There were no mortalities and no adverse treatment-related clinical signs. No test item related effects on gross pathology or body weights were observed. A statistically significant decrease in heart weight was observed in the interim male high-dose group compared to the saline- control group. This change was minor in magnitude and considered to have no toxicological significance. The test item did not cause any overt adverse histomorphological findings and microscopic examination of the injection site indicated that the local tolerability of the test item did not differ significantly from that of physiological saline. There were statistically significant changes in the test item-treated groups compared to the saline or vehicle groups in some of the clinical pathology parameters. These changes were without any adverse biological consequence and did not correlate with any histomorphological findings. These changes were therefore regarded to be of no toxicological significance.

To confirm that correct dose test articles were given to the animals, samples of the dosing solution were analyzed for actual concentrations and purity of the test substance ([1-^{13}C]pyruvate), and in addition pH and osmolality were measured. Selected samples were analyzed for concentration of AH111501 by HPLC and purity of [1-^{13}C]pyruvate by NMR. All analytical results were within the expected range.

No-Observed-Adverse-Effect-Level (NOAEL) in this study was 892 mg/kg bw for [1-^{13}C]pyruvate and 17 mg/kg bw for AH111501 in TRIS/EDTA dissolution medium. The NOAEL expressed as Human Equivalent Doses (HED) based on body surface area was 144 mg/kg bw for [1-^{13}C]pyruvate and 2.75 mg/kg bw for AH111501.

5.3.2.3 AH112623 (Parapyruvate)

5.3.2.3.1 GE study number: B101044. An Expanded Acute Dose Toxicity Study with Intravenously Injected Parapyruvate in Male and Female Rats (GLP)

Four groups of 12 male and 12 female Sprague-Dawley rats were administered either saline (control animals), or a high dose (1216 mg/kg bw) of parapyruvate (AH112623) dissolved in 20 mM phosphate buffer containing 75 mM NaOH. One group of 6 male and 6 female Sprague-Dawley rats was administered a low dose of parapyruvate (608 mg/kg bw). To obtain maximum exposure, the animals were dosed twice on the same day (with approximately 4 h between the administrations)

with the maximum recommended dose volume of 20 ml/kg for the high-dose and the control groups and with 10 ml/kg for the low-dose group. To study acute effects, an interim kill on six males and six females in the high-dose and the control groups was performed at 24 h after dosing. Fourteen days post-treatment, the remaining animals were killed and necropsied.

There were no mortalities and no adverse treatment-related clinical signs. No test item related effects on gross pathology or body weights were observed. A small but statistically significant decrease in absolute liver weight was seen for the male high dose group at 24 h after dosing compared to the saline group. This did not correlate to any histomorphological findings and the change was of no toxicological relevance. There were no test item related microscopic findings in this study in either the interim group or the terminal group. All microscopic findings were either related to venipuncture or were incidental and of the type routinely observed in Sprague-Dawley rats of this age. There were small but statistically significant changes for some of the clinical pathology parameters when the test item treated groups were compared to the saline group, however, these changes did not correlate with any histomorphological findings and were of no toxicological relevance.

To confirm that correct dose test articles were given to the animals, samples of the dosing solution were analyzed for concentrations and purity of parapyruvate by NMR. In addition, pH and osmolality were measured. The variability of the analytical results was considered acceptable. However, the lowest calculated assay value for parapyruvate was 30.4 mg/ml, which was 76% of the target value of 40 mg/ml. Therefore, the estimated actual maximum dose of parapyruvate administered to the animals was estimated as 1216 mg/kg bw.

Intravenous administration of a dose of 1216 mg/kg bw parapyruvate to rats was well tolerated when divided in two intravenous administrations of 20 ml/kg separated by 4 h and injected at a rate of 1.2 ml/min. The estimated No-Observed-Adverse-Effect-Level (NOAEL) in this study was 1216 mg/kg bw for parapyruvate. The NOAEL expressed as Human Equivalent Doses (HED based on body surface area was 196 mg/kg bw.

5.3.2.4 AH112615 (Reaction-product between pyruvic acid and TRIS)

5.3.2.4.1 GE study number: B101046. An expanded acute dose toxicity study with intravenously injected AH-112615 (PA-TRIS) in male and female rats (GLP)

Six groups of 12 male and 12 female Sprague-Dawley rats were administered either saline control, a high dose (1400 mg/kg bw) or a low dose (700 mg/kg bw) of test item AH112615 dissolved in sterile water. To obtain maximum exposure, the animals were dosed twice on the same day (with approximately 4 h between the administrations) with the maximum recommended dose volume of 20 ml/kg for the high-dose and the control groups and with 10 ml/kg for the low-dose group. To study acute effects, an interim kill was performed on half of the animals 24 h after dosing. Fourteen days post-treatment, the remaining animals were killed and necropsied.

There were no mortalities and no adverse treatment-related clinical signs. No test item related effects on gross pathology or body weights were observed. A small but statistically significant decrease in liver weight in the interim female high dose group and a small but statistically significant increase in brain weight in the terminal female high dose group were found compared to the saline group. These changes did not correlate with any histomorphological findings and were of no toxicological relevance. There were no test item related microscopic findings in this study in either the interim-kill group or the terminal-kill group. All microscopic findings were either related to venipuncture or were incidental and of the type routinely observed in Sprague-

Dawley rats of this age. There were small but statistically significant changes in the test item-treated groups compared to the saline group in some of the clinical pathology parameters. However, these changes did not correlate with any histomorphological findings and were of no toxicological relevance.

Samples of the dosing solution were analyzed for osmolality and pH. The analytical results were as expected and considered acceptable. No analysis of the concentration AH112615 was done. Therefore, the nominal concentration was used to estimate the dose of AH112615 administered to the animals.

Intravenous administration of a nominal dose of 1400 mg/kg bw of AH112615 to rats was well tolerated when divided in two intravenous administrations of 20 ml/kg separated by 4 h and injected at a rate of 1.2 ml/min. The nominal No-Observed-Adverse-Effect-Level (NOAEL) in this study was 1400 mg/kg bw for AH112615. The NOAEL expressed as Human Equivalent Doses (HED based on body surface area was 226 mg/kg bw.

5.3.2.5 AH113462 (Lactone)

5.3.2.5.1 Covance study number 2789/011 (GE study number: B101065) Parapyruvate/Lactone in TRIS Solution: Expanded acute dose toxicity study in the rat (GLP)

The lactone impurity tested in this study may be present under the acidic conditions of the Kit Component 1 (Mixture of [^{13}C]pyruvic acid and 15 mM AH111501 sodium salt) but is under neutral conditions converted to parapyruvate. The inter-conversion takes some minutes, and lactones may therefore be present in Hyperpolarized Pyruvate (^{13}C) Injection, as this is injected shortly after neutralization. The test article used in the present study consisted of parapyruvate formulated with HCl. The acidic mixture was allowed to equilibrate for approximately 48 h after which it was neutralized with TRIS neutralization solution and administered within 10 min of neutralization. This test article procedure ensured that the lactone was present in the highest achievable concentration while providing a test item suitable for intravenous administration. The two potential impurities, parapyruvate and lactone, were present in similar concentration in the neutralized test article.

Four groups, each of six male and six female Crl:WI (Han) rats, were given a single intravenous administration of 0.9% saline, TRIS vehicle control, and nominal doses of 100 (low dose) or 200 (high dose) mg/kg bw parapyruvate/Lactone in Tris Solution. An interim kill was performed on half of the animals 24 h after dosing. Fourteen days post-treatment, the remaining animals were killed and necropsied.

Parapyruvate/lactone in TRIS solution was formulated according to the user instruction, assuming the stock solution comprised concentrations of 10 mg/ml for each of main components, parapyruvate and lactone. However, the certificate of analysis showed that the stock solution contained 8.6 mg/ml parapyruvate and 8.19 mg/ml lactone. The difference between the target assay value and the observed assay value was attributed to increased water content in the dry dispensed substance. Accordingly, the actual concentrations for parapyruvate and lactone were approximately 14-18% less than expected.

Rats were evaluated for in-life observations, bodyweight, hematology, clinical chemistry, organ weights, macroscopic observations and microscopic observations.

Dose administration of parapyruvate/Lactone in TRIS Solution at nominal dose levels of 100 and

200 mg/kg was well tolerated, with no clinical observations, effects on organ weights, or adverse effects on body weight and clinical pathology parameters.

Histopathological evaluations concluded that there were no changes that were related to treatment with the test item. The possible exception to this was the increased incidences of minimal inflammatory foci in the liver of the top dose males at 14 days post- treatment, and in the top dose females at 24 h and 14 days after dosing, but the minor nature of these lesions, together with the occurrence of this finding in two control females at 14 days post-treatment, indicates that it is likely to be spontaneous, i.e. not related to treatment.

In conclusion, administration of parapyruvate/lactone in TRIS Solution at nominal dose levels of 100 and 200 mg/kg bw was well tolerated, with no clinical observed effects, effects on organ weights, or adverse effects on body weight and clinical pathology parameters. The NOAEL for parapyruvate/lactone in TRIS Solution was 172 mg parapyruvate/kg bw and 164 mg lactone/kg bw.

5.3.3 Repeat-Dose Toxicity

No studies have been performed to date.

5.3.4 Genotoxicity

5.3.4.1 [1-^{13}C]pyruvate and AH111501:

5.3.4.1.1 TNO study number: 5605/15 (GE study number: B101014). Bacterial reverse mutation test with AH111501 dissolved in 13C AH110896 (GLP)

The test item was assayed for mutagenicity in five histidine-requiring strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, TA102), both in the absence and in the presence of S-9 (rat liver metabolic activation system), in two separate experiments. The contents of the vial containing a mixture of [1-^{13}C]pyruvic acid and AH111501 were dissolved in 44 ml TRIS/EDTA dissolution medium, so the nominal composition of the test item stock solution was 50 mg/ml [1-^{13}C]pyruvate and 0.97 mg/ml AH111501. The amounts tested ranged from 61 to 5000 µg/plate with respect to [1-^{13}C]pyruvate.

In all strains, both in the presence and absence of S-9, the test article of [1-^{13}C]pyruvate containing AH111501 did not cause a dose-related or more than two-fold increase in the mean number of revertant colonies appearing in the test plates when compared to the background spontaneous reversion rate observed with the vehicle. The mean number of his+ revertant colonies with the vehicle controls was within the acceptable range, and the positive controls gave the expected increase in the mean number of revertant colonies.

In conclusion, mixture of [1-^{13}C]pyruvate and AH111501 dissolved in TRIS/EDTA dissolution medium did not induce mutation in the five histidine-requiring strains (TA98, TA100, TA1535, TA1537, TA102) of *Salmonella typhimurium*, when tested at amounts up to 5000 µg/plate with respect to [1-^{13}C]pyruvate in the presence and absence of S-9.

5.3.4.1.2 Covance study number: 2570/3 (GE study number: B101043). Formulation containing 557 mM AH-111501 and 500 mM 13C Pyruvate (AH-110896) in 200 mM TRIS: Reverse Mutation in five Histidine-requiring strains of Salmonella typhimurium (GLP)

A test article containing 500 mM [1-^{13}C]pyruvate and 0.85 mg/ml AH111501 in TRIS/EDTA dissolution medium was assayed for mutagenicity in five histidine-requiring strains (TA98, TA100, TA1535, TA1537 and TA102) of *Salmonella typhimurium*, both in the absence and in the presence of S-9 (rat liver metabolic activation system), in two separate experiments using treatment amounts of [1-^{13}C]pyruvate ranging from 1.6 to 5352 μg/plate.

Dose test article analysis confirmed correct test article of test item. No statistically significant dose-related and reproducible increases in number of revertant colonies were observed in any strain following treatments in the absence or presence of S-9. Therefore, this study did not provide any evidence of any mutagenic activity of [1-^{13}C]pyruvate. It was concluded that the [1-^{13}C]pyruvate did not induce mutations in five histidine-requiring strains (TA98, TA100, TA1535, TA1537 and TA102) of *Salmonella typhimurium* when tested under the conditions of the study; i.e. up to 5352 μg/plate of [1-^{13}C]pyruvate, in the absence or presence of S-9.

5.3.4.1.3 TNO study number: 5603/08 (GE study number: B101015). Gene mutation test at the TK-locus of L5178Y cells with AH111501 dissolved in 13C AH110896 (GLP)

The test item was assayed for its ability to induce mutation at the *tk* locus (5 trifluorothymidine resistance) in mouse lymphoma cells. The study consisted of two independent experiments, each in the absence and presence of S-9 (rat liver metabolic activation system). The contents of the vial containing a mixture of [1-^{13}C]pyruvic acid and AH111501 were dissolved in 44 ml TRIS/EDTA dissolution medium, so the nominal composition of the test item stock solution was 50 mg/ml [1-^{13}C]pyruvate and 0.97 mg/ml AH111501. The experiments were conducted with concentrations ranging from 9.2 μg/ml to 5000 μg/ml of [1-^{13}C]pyruvate. A TRIS/EDTA vehicle control was included in all experiments. A 4 h treatment incubation period was used in all experiments performed in the presence of S-9. In the absence of S-9, a 24 h treatment incubation period was used.

In the first experiment, an increase of about 200 mutants per 1 million clonable cells compared to the vehicle control solution was observed at the highest concentration of 5000 μg/ml [1-^{13}C]pyruvate in the absence of S-9. The mutant frequency (MF) of the vehicle control was also increased compared to that of the historical negative control. In the presence of S-9, no increases in MF were observed at any concentration of the test item.

In the second experiment in the absence of S-9, an untreated control (culture medium), a mannitol control solution with identical osmolality to 5000 μg/ml of AH110896, and a pH-neutralized TRIS/EDTA dissolution medium control solution were included. In the absence of S-9, an increase in MF compared to the vehicle control solution was observed at the highest concentration of 5000 μg/ml [1-^{13}C]pyruvate. The MF of the vehicle control was also increased compared to the normal control. The MF of the mannitol control was in the same range as the normal control. However, the MF of the neutralized TRIS/EDTA dissolution medium was highest of all. In the presence of S-9 no increases were observed at any concentration of the test item.

In the presence of S-9, the test article containing [1-^{13}C]pyruvate and AH111501 was not mutagenic in this assay. In the absence of S-9, the vehicle control induced a two to three-fold

increase in MF in this assay. Treatment with the highest concentration of test item (5000 μg/ml [1-^{13}C]pyruvate) in the absence of S-9 doubled the mutation frequency compared to vehicle-treated cells, but no increase was seen at lower concentrations tested. Due to the non-physiological conditions in this *in vitro* experiment when high concentrations of the test item are tested, the significance of this finding was considered as equivocal.

5.3.4.1.4 Covance study number: 2570/4 (GE study number: B101045), Formulation containing 557 μM AH-111501 and 500 mM ^{13}C Pyruvate (AH-110896) in 200 mM TRIS: Mutation at the thymidine kinase (*tk*) locus of mouse lymphoma L5178Y cells (MLA) using the Microtitre^{R} fluctuation technique (GLP)

A test article containing 500 mM [1-^{13}C]pyruvate and 0.85 mg/ml AH111501 in TRIS/EDTA dissolution medium was assayed for its ability to induce mutation at the *tk* locus in mouse lymphoma cells (rendering the cells resistant to 5 trifluorothymidine [TFT] toxicity) using a fluctuation protocol. The study consisted of a cytotoxicity range-finding experiment followed by two independent experiments, each conducted in the absence and presence of S-9 (rat liver metabolic activation system) with concentrations of [1-^{13}C]pyruvate ranging from 62.5 to 5018 μg/ml. A 3 h treatment incubation period was used for all experiments performed in the presence of S-9. In the absence of S-9, the range finder was performed using both a 3 h and a 24 h treatment incubation. Experiment 1 was performed using a 3 h treatment incubation and Experiment 2 was performed using a 24 h treatment incubation.

Dose test article analysis confirmed correct test article of test item. When the test article was tested at [1-^{13}C]pyruvate concentrations up to 5018 μg/ml in the absence of S-9 in Experiment 1, significant increases in mutant frequency were observed at the three highest concentrations tested (3000 to 5018 μg/ml). The increases were observed at concentrations where increases in osmolality of >50 mOsm/kg (compared to concurrent vehicle controls) were seen. When tested up to toxic concentrations in Experiment 2, increases in mutant frequency were observed at the 5 highest [1-^{13}C]pyruvate concentrations tested (1200 to 2000 μg/ml). The increases in mutant frequency observed in Experiment 2 were not associated with large increases in osmolality (<50 mOsm/kg, compared to concurrent vehicle controls). In the presence of S-9 when tested up to 5018 μg/ml, increases in mutant frequency were observed at the two highest [1-^{13}C]pyruvate concentrations tested in Experiment 1 (4000 and 5018 μg/ml) and at the highest concentration tested in Experiment 2 (5018 μg/ml). The increases in mutant frequency were observed at concentrations where increases in osmolality of >50 mOsm/kg (compared to concurrent vehicle controls) were also seen. However, large increases in osmolality were observed at 3000 μg/ml and above in both experiments and were not always associated with marked increases in mutant frequency. Therefore, the increases in osmolarity could not have been the sole factor responsible for the significant increases in mutant frequency. Highly significant linear trends for the concentration-effect relationships were observed following treatments in the absence and presence of S-9 in Experiments 1 and 2.

It was concluded that the test item induced mutation at the *tk* locus of L5178Y mouse lymphoma cells when tested under the conditions employed in this study. These conditions included treatments up to toxic concentrations for 24 h in the absence of S-9 and up to approximately 5000 μg/ml for 3 h in the absence and presence of S-9. The mutagenic activity following 3 h treatments in the absence and presence of S-9 was observed at concentrations at which marked increases in osmolality were also seen.

5.3.4.1.5 TNO study number: 5604/04 (GE study number: B101016). Micronucleus test in bone marrow cells of rats treated intravenously with AH111501 dissolved in ^{13}C AH110896 (GLP)

The test item containing 44.6 mg/ml [1-^{13}C]pyruvate and 0.85 mg/ml AH111501 in TRIS/EDTA dissolution medium was examined for its mutagenic potential in a bone marrow micronucleus test in male rats. The micronucleus test was performed with male rats only and with the highest possible dose level of 1784 mg/kg bw of [1-^{13}C]pyruvate and 34 mg/kg bw of AH111501. The dosing was divided into two intravenous injections of 20 ml/kg bw each separated by an interval of approximately 24 h. The injection volume of 20 ml/kg bw as an upper limit for intravenous administration to rats, was based on a generally accepted recommendation [Diehl et al. 2001]. Seven male rats (including two reserve rats) were treated with [1-^{13}C]pyruvate and AH111501. The negative control group, consisting of five male rats, was treated in a similar way with saline (0.9% sodium chloride). The positive control group, consisting of five male rats, was given a single intraperitoneal injection with the mutagen mitomycin C (0.75 mg/kg bw). At 24 h after the final treatment, all rats were sacrificed. Bone marrow cells were collected from both femurs of each animal and pooled and processed into smears for microscopic examination.

The incidences of micronucleated polychromatic erythrocytes per 2000 polychromatic erythrocytes in male rats treated with [1-^{13}C]pyruvate and AH111501 were not statistically significantly different from those found in the negative (saline) controls. Therefore, this study indicates that treatment with [1-^{13}C]pyruvate and AH111501 did not result in genotoxicity to bone marrow cells. The positive control group differed statistically significantly from the negative control group ($p<0.001$), demonstrating the sensitivity of the test system. The number of polychromatic erythrocytes per 200 erythrocytes in male rats treated with [1-^{13}C]pyruvate and AH111501 was not statistically significantly different from that found in the negative (saline) controls. This indicates that treatment with [1-^{13}C]pyruvate and AH111501 did not result in cytotoxicity to bone marrow cells.

These data support the conclusion that, at the doses applied under the conditions used in this study, the test item [1-^{13}C]pyruvate and AH111501 did not produce chromosomal damage or damage to the mitotic spindle apparatus in bone marrow cells of male rats treated with 892 mg pyruvate/kg bw/day and 17 mg/kg bw/day AH111501 on two consecutive days, when analyzed 24 h after the final dose administration.

5.3.4.1.6 Covance study number: 2789/6 (GE study number: B101057) Detection of DNA damage in the blood of treated rats using the Comet assay (GLP)

In this study, the potential of the mixture of 44.6 mg/ml [1-^{13}C]pyruvic acid and 0.85 mg/ml AH111501 sodium salt dissolved in TRIS/EDTA dissolution medium to induce DNA strand breaks or alkali labile sites was investigated by assessing the extent of DNA damage in blood of treated rats. To obtain maximum exposure, the animals were dosed twice on the same day (with approximately 5 h between the administrations), with the maximum recommended dose volume of 20 ml/kg bw (892 mg pyruvate/kg bw), on two consecutive days. The highest dose to be tested was determined in a range-finder in three male rats. Groups of six male rats were administered either test article at 1784 mg pyruvate/kg bw/day or the test article diluted 1:1 with sterile water at doses of 892 mg pyruvate/kg bw/day. The test article was administered via intravenous infusion at a rate of 1.2 ml/min. The blood of the treated rats was analyzed for DNA damage 3 h after the final dose administration. The volume control in the study was 0.9% saline. Animals

were dosed using the same dosing regimen and dose volume as that used for test article-treated animals. Ethyl methanesulfonate (EMS), the positive control, was dissolved in purified water and administered orally by gavage as a single dose at 250 mg/kg bw (dose volume of 10 ml/kg bw) to a group of six rats which were killed 3 h after dose administration.

In the range-finder one rat displayed gasping and all three animals were lethargic on Day 2. There were no observations on Day 3 and 4. In the Comet study one animal dosed with saline died immediately following its fourth dose, and one test article treated animal showed ventral staining following the fourth administration. No other clinical signs of toxicity were observed in any animal following treatments with the test article or EMS.

The mean tail moment and tail intensity for the saline control group was like the laboratories validation data. Mean tail moment and tail intensity values for the positive control group exhibited a statistically significant increase over concurrent solvent controls. The study was therefore accepted as valid. The supplementary cytotoxicity data ('cloud' assessment and diffusion slide analysis) for blood from animals treated with the test article were considered acceptable as demonstrated by low levels of cytotoxicity, necrosis or apoptosis. Comet analysis of blood provided tail moment and tail intensity values for the test article treated animals that were considered consistent with the concurrent saline control group. No increases or decreases in tail moment or tail intensity in test article treated groups sufficient in magnitude to be considered indicative of any cross-linking or DNA damage effects were observed.

It is concluded that, under the conditions of this Comet assay, the mixture of 44.6 mg/ml [1-^{13}C]pyruvic acid and 0.85 mg/ml AH111501 sodium salt dissolved in TRIS/EDTA dissolution medium did not induce DNA damage in the blood of rats treated with up to 1784 mg pyruvate/kg bw/day and 34 mg/kg bw/day AH111501 on two consecutive days, when analyzed 3 h after the final dose administration.

5.3.4.2 AH112623 (Parapyruvate)

5.3.4.2.1 Covance study number: 2570/5 (GE study number: B101042) Parapyruvate: Reverse mutation in five histidine-requiring strains of *Salmonella typhimurium* (GLP)

Parapyruvate was assayed for mutagenicity in five histidine-requiring strains (TA98, TA100, TA1535, TA1537 and TA102) of *Salmonella typhimurium*, both in the absence and in the presence of S-9 (rat liver metabolic activation system), in two separate experiments.
Parapyruvate was dissolved in sterile water to give final nominal amounts of parapyruvate ranging from 1.6 to 5000 µg/plate. Negative and positive controls were included in the assay.

Test item analysis demonstrated an actual concentration of 44.9 mg/ml parapyruvate in the stock test article (90% of target). No statistically significant concentration-related and reproducible increases in numbers of revertant colonies were observed in any strain following treatments with test item in the absence or presence of S-9. It was concluded that parapyruvate did not induce mutations in five histidine-requiring strains (TA98, TA100, TA1535, TA1537 and TA102) of *Salmonella typhimurium* when tested under the conditions of this study. These conditions included treatments at concentrations up to 4490 µg/plate, in the absence and in the presence of S-9.

5.3.4.2.2 Covance study number: 2570/6 (GE study number: B101041) Parapyruvate: Mutation at the Thymidine Kinase (*tk*) Locus of Mouse Lymphoma L5178Y Cells (MLA) using the Microtitre^{R} Fluctuation Technique (GLP)

Parapyruvate was assayed for its ability to induce mutation at the *tk* locus in mouse lymphoma cells (rendering the cells resistant to 5 trifluorothymidine [TFT] toxicity) using a fluctuation protocol. The study consisted of a cytotoxicity range-finding experiment followed by two independent experiments, each conducted in the absence and presence of S-9 (rat liver metabolic activation system) using nominal treatment concentrations ranging from 156 to 5000 µg/ml. A 3 h treatment incubation period was used for all experiments performed in the presence of S-9. In the absence of S-9, the range finder was performed using both a 3 h and a 24 h treatment incubation, Experiment 1 was performed using a 3 h treatment incubation and Experiment 2 was performed using a 24 h treatment incubation.

Test item analysis demonstrated an actual concentration of 45.6 mg/ml parapyruvate in the stock test article (91% of target).

In Experiment 1 and 2 (3 h treatments) a weak linear trend was observed in the absence and presence of S-9. However, no marked increases in mutant frequency were observed with any 3 h treated cultures and therefore this result was not considered biologically significant.

In Experiment 2 (24 h treatment without S-9), significant increases in mutant frequency were observed at nominal concentrations of 3500 and 4000 µg/ml (maximum concentration limited by toxicity). These increases also showed a significant linear trend. It was noted that these increases were observed for treatment cultures where increases in osmolality of 63 and 86 mOsm/kg above the concurrent negative control occurred. It is known that changes in osmolality of more than 50 mOsm/kg can be responsible for increases in mutant frequencies, and from these study data it cannot be established whether these results are due to true mutation induction or epigenetic effects of increases in osmolality.

It was concluded that parapyruvate did not induce mutation at the tk locus of L5178Y mouse lymphoma cells when tested under the 3 h treatment conditions employed in this study. These conditions included treatments up to a concentration of 4550 µg/ml in two independent experiments, in the absence and presence of S-9. However, it was concluded that parapyruvate may induce mutation at the *tk* locus of L5178Y mouse lymphoma cells when tested under the 24 h treatment conditions in the absence of S-9 up to toxic concentrations (3640 µg/ml). As increases in mutant frequency were only noted at concentrations where marked changes in osmolality occurred, it cannot be determined from these data whether the results are due to true mutation induction or epigenetic effects of increases in osmolality.

5.3.4.3 AH112615 (Reaction-product between pyruvic acid and TRIS)

5.3.4.3.1 Covance study number: 2570/7 (GE study number: B101047). AH-112615: Reverse mutation in five histidine-requiring strains of *Salmonella typhimurium* (GLP)

AH112615 was assayed for mutagenicity in five histidine-requiring strains (TA98, TA100, TA1535, TA1537 and TA102) of *Salmonella typhimurium*, both in the absence and in the presence of S-9 (rat liver metabolic activation system), in two separate experiments.
AH112615 was dissolved in sterile water and nominal amounts of AH112615 ranging from 1.6 to 5000 µg/plate were tested. Negative and positive controls were included in the assay.

No statistically significant, concentration-related and reproducible increases in number of revertant colonies were observed following treatment of any strain in the absence or presence of S-9. Therefore, this study did not provide any evidence of mutagenic activity of AH112615. It was concluded that AH112615 did not induce mutations in five histidine-requiring strains (TA98, TA100, TA1535, TA1537 and TA102) of *Salmonella typhimurium* when tested under the conditions of this study. These conditions included treatments at nominal amounts up to 5000 µg/plate, in the absence and in the presence of S-9.

5.3.4.3.3 Covance study number: 2570/8 (GE study number: B101048). AH-112615: Mutation at the Thymidine Kinase (*tk*) Locus of Mouse Lymphoma L5178Y Cells (MLA) using the Microtitre^{R} Fluctuation Technique (GLP)

AH112615 was assayed for its ability to induce mutation at the *tk* locus in mouse lymphoma cells (rendering the cells resistant to 5 trifluorothymidine [TFT] toxicity) using a fluctuation protocol. The study consisted of a cytotoxicity range-finding experiment followed by two independent experiments, each conducted in the absence and presence of S-9 (rat liver metabolic activation system) using nominal treatment concentrations ranging from 125 to 5000 µg/ml. A 3 h treatment incubation period was used for all experiments performed in the presence of S-9. In the absence of S-9, the range-finder was performed using a 3 h and a 24 h treatment incubation period, Experiment 1 was performed using a 3 h treatment incubation and Experiment 2 was performed using a 24 h treatment incubation.

In Experiments 1 and 2, weak linear trends were observed in the presence of S-9 in Experiment 1 (3 h) and in the absence of S-9 in Experiment 2 (24 h). No marked increases in mutant frequency were observed in either experiment. Therefore, these observations were not considered biologically significant.

It is concluded that AH112615 did not induce mutation at the *tk* locus of L5178Y mouse lymphoma cells when tested under the conditions employed in this study. These conditions included treatments at nominal concentrations of up to 5000 µg/ml in two independent experiments in the absence and presence of S-9.

5.3.4.4 AH111501 (EPA)

5.3.4.4.1 Covance study number: 2570/10 (GE study number: B101055). AH111501: Reverse mutation in five histidine-requiring strains of *Salmonella typhimurium* (GLP)

AH111501 was assayed for mutation in five histidine-requiring strains (TA98, TA100, TA1535, TA1537 and TA102) of *Salmonella typhimurium*, both in the absence and in the presence of S-9 (rat liver metabolic activation system), in two separate experiments AH111501 was dissolved in sterile water and nominal amounts of AH111501 ranging from 1.6 to 5000 µg/plate were tested. Negative and positive controls were included in the assay.

No statistically significant, concentration-related and reproducible increases in number of revertant colonies were observed following any strain treatments in the absence or presence S-9. Therefore, this study did not provide any evidence of mutagenic activity of AH111501. It was concluded that AH111501 did not induce mutation in five histidine-requiring strains (TA98, TA100, TA1535, TA1537 and TA102) of *Salmonella typhimurium* when tested under the conditions of this study. These conditions included treatments at amounts up to 5000 µg/plate, in the absence and in the presence of S-9.

5.3.4.4.2 Covance study number: 2570/11 (GE study number: B101056) Mutation at the Thymidine Kinase (*tk*) Locus of Mouse Lymphoma L5178Y Cells (MLA) using the Microtitre^{R} Fluctuation Technique (GLP)

AH111501 was assayed for its ability to induce mutation at the *tk* locus in mouse lymphoma cells (rendering the cells resistant to 5 trifluorothymidine [TFT] toxicity) using a fluctuation protocol. The study consisted of a cytotoxicity range-finding experiment followed by two independent experiments, each conducted in the absence and presence of S-9 (rat liver metabolic activation system) using nominal treatment concentrations ranging from 500 to 5000 µg/ml. A 3 h treatment incubation period was used for all experiments performed in the presence of S-9. In the absence of S-9, the range-finder was performed using a 3 h and a 24 h treatment incubation, Experiment 1 was performed using a 3 h treatment incubation and Experiment 2 was performed using a 24 h treatment incubation.

No marked increases in mutation frequencies were observed with any of the 3 h exposure periods (with and without S-9 in Experiment 1 and with S-9 in Experiment 2).

Treatment with AH111501 up to 5000µg/ml in Experiment 2 in the absence of S-9 (24 h treatment) caused a dose-dependent increase in the mutation frequency. The linear trend for the dose-relationship was statistically significant, but the mutation frequency did not exceed the Global Evaluation Factor (GEF) for the assay and was therefore not considered to be significant. When the experiment was repeated the linear trend for a dose-dependent increase in mutation frequency was again statistically significant. The increases were even smaller than in the original 24 h experiment, but also the positive control responses were small.

It was concluded that AH111501 did not induce mutation at the *tk* locus of L5178Y mouse lymphoma cells when tested under the 3 h treatment conditions employed in this study. These conditions included treatments with nominal concentrations of up to 5000 µg/ml in two independent experiments, in the absence and presence of S-9. However, it was concluded that AH111501 induces mutation at the tk locus of L5178Y mouse lymphoma cells following 24 h treatment in the absence of S-9 but that this response is very weak.

5.3.4.5 AH113462 (Lactone)

The lactone impurity may be present under the acidic conditions of the Kit Component 1 (mixture of [^{13}C]pyruvic acid and 15 mM AH111501 sodium salt) but is converted under neutral conditions to parapyruvate. The inter-conversion takes some minutes, and lactones may therefore be present in Hyperpolarized Pyruvate (^{13}C) Injection, as this is injected shortly after neutralization. For the *in vitro* genotoxicity studies, a test article of lactone was used which consisted of parapyruvate formulated with HCl, the acidic mixture was allowed to equilibrate for approximately 48h before it was used. The two potential impurities parapyruvate and lactone were present in similar concentration in this test item; Parapyruvate/lactone in HCl solution. In the *in vivo* genotoxicity study of induction of micronuclei in the bone marrow in treated rats, the test article consisted of parapyruvate formulated with HCl, the acidic mixture was allowed to equilibrate for approximately 48h after which it was neutralized with TRIS neutralization solution and administered within 10 min of neutralization. This test article procedure ensured that the lactone was present in the highest achievable concentration while providing a test item suitable for intravenous administration.

The two potential impurities parapyruvate and lactone were present in similar concentration in the

neutralized test article parapyruvate/lactone in TRIS solution.

5.3.4.5.1 Covance study number: 2789/8 (GE study number: B101062) Reverse mutation in five histidine-requiring strains of *Salmonella typhimurium* (GLP)

Parapyruvate/lactone in HCl solution was assayed for mutation in five histidine-requiring strains (TA98, TA100, TA1535, TA1537 and TA102) of *Salmonella typhimurium*, both in the absence and in the presence of metabolic activation by an Aroclor 1254-induced rat liver post mitochondrial fraction (S-9), in two separate experiments.

Parapyruvate/lactone in HCL solution was formulated according to the user instruction, assuming the stock solution comprised concentrations of 50 mg/ml for each of the main components, parapyruvate and lactone. However, the certificate of analysis showed that the stock solution contained 39.53 mg/ml parapyruvate and 40.78 mg/ml lactone. The difference between the target assay value and the observed assay value was attributed to increased water content in the dry dispensed substance. Accordingly, the actual concentrations for parapyruvate and lactone were approximately 18-21% less than expected.

In Experiment 1, treatment of all the test strains was performed in the absence and in the presence of S-9, using final concentrations of parapyruvate/lactone in HCl solution at 0.32, 1.6, 8, 40, 200, 1000, and 5000 μg/plate, plus negative (vehicle) and positive controls. Following these treatments, evidence of toxicity was observed at 5000 μg/plate in all strains in the absence and presence of S-9.

In Experiment 2, treatment of all the tester strains was performed in the absence and in the presence of S-9 with a maximum test concentration of 5000 μg/plate. Narrowed concentration ranges were employed (20.48 – 5000 μg/plate), in order to examine more closely those concentrations of parapyruvate/Lactone in HCl solution approaching the maximum test concentration and therefore considered most likely to provide evidence of any mutagenic activity. In addition, all treatments in the presence of S-9 were further modified by the inclusion of a pre-incubation step. In this way, it was hoped to increase the range of mutagenic chemicals that could be detected using this assay system. Following these treatments, evidence of toxicity was observed at 2000 μg/plate and above in strains TA1537 and TA102 in the absence of S-9, at 5000 μg/plate in strains TA98 and TA100 in the absence and presence of S- 9, and in strains TA1535, TA1537 and TA102 in the presence of S-9 only.

It was concluded that parapyruvate/lactone in HCl solution did not induce mutation in five histidine-requiring strains (TA98, TA100, TA1535, TA1537 and TA102) of *Salmonella typhimurium* when tested under the conditions of this study. These conditions included treatments at nominal concentrations up to 5000 μg/plate, in the absence and in the presence of a rat liver metabolic activation system (S-9).

5.3.4.5.2 Covance study number: 2789/9 (GE study number: B101063) Parapyruvate/lactone in HCl solution: Mutation at the thymidine kinase (*tk*) locus of mouse lymphoma L5178Y cells (MLA) using the Microtitre^{R} fluctuation technique

Parapyruvate/lactone in HCl solution was assayed for its ability to induce mutation at the *tk* locus (5-trifluorothymidine [TFT] resistance) in mouse lymphoma cells using a fluctuation protocol. The study consisted of a cytotoxicity Range-Finder Experiment followed by two independent

experiments, each conducted in the absence and presence of metabolic activation by an Aroclor 1254 induced rat liver post-mitochondrial fraction (S-9).

Parapyruvate/lactone in HCL solution was formulated according to the user instruction, assuming the stock solution comprised concentrations of 50 mg/mL for each of main components, parapyruvate and lactone. However, the certificate of analysis showed that the stock solution contained 39.53 mg/ml parapyruvate and 40.78 mg/ml lactone. The difference between the target assay value and the observed assay value was attributed to increased water content in the dry dispensed substance. Accordingly, the actual concentrations for parapyruvate and lactone were approximately 18-21% less than expected.

A 3 h treatment incubation period was used for all experiments performed in the presence of S-9. In the absence of S-9, the Range-Finder Experiment was performed using 3 h and 24 h treatment incubation periods. Experiment 1 was performed using a 3 h treatment incubation and Experiment 2 was performed using a 24 h treatment incubation.

In the cytotoxicity Range-Finder Experiment, 3 h treatment, six concentrations were tested, in the absence and presence of S-9, ranging from 156.3 to 5000 μg/ml (an acceptable maximum concentration for *in vitro* genetic toxicology studies according to current regulatory guidelines). The highest concentration to provide >10% relative total growth (RTG) was 1250 μg/mL, which gave 14% and 48% RTG in the absence and presence of S-9, respectively.

In the cytotoxicity Range-Finder Experiment, 24 h treatment, nine concentrations were tested in the absence of S-9, ranging from 19.53 to 5000 μg/ml. The highest concentration to provide >10% RTG was 625 μg/ml, which gave 41% RTG.

Accordingly, for Experiment 1, ten concentrations, ranging from 200 to 1600 μg/ml in the absence of S-9 and from 250 to 2500 μg/ml in the presence of S-9, were tested. Two days after treatment, the highest concentrations analyzed to determine viability and TFT resistance were 1400 μg/ml in the absence of S-9 and 1250 μg/ml in the presence of S-9, which gave 8% and 37% RTG, respectively. In the presence of S-9, a steep concentration-related increase in toxicity was observed between 1250 and 1500 μg/ml, giving 37% and 3% RTG, respectively, and there was marked heterogeneity between cultures at the higher concentration. Cultures were initially analyzed for mutation at 1500 μg/ml, but the mutation data were considered extremely unreliable due to the high cytotoxicity and marked heterogeneity.

In Experiment 2, ten concentrations ranging from 125 to 1200 μg/ml in the absence of S-9 (24-hour treatment) and from 250 to 1750 μg/ml in the presence of S-9 (3 h treatment), were tested. Two days after treatment, the highest concentrations analyzed to determine viability and TFT resistance were 750 μg/ml in the absence of S-9 and 1300 μg/ml in the presence of S-9, which gave 14% and 24% RTG, respectively. As in Experiment 1, these small increases in mutation frequency were seen under conditions of reduced pH, increased osmolality and at cytotoxic concentrations.

In Experiment 1 in the absence of S-9, an increase in mean mutant frequency of approximately 148 (thus exceeding the Global Evaluation Factor [GEF] of 126 mutants per 10^6 viable cells) was observed at 1400 μg/ml (giving 8% RTG) and there was a weak linear trend. However, a steep concentration-related increase in toxicity was observed between 1250 and 1400 μg/ml (giving 46% and 8% RTG, respectively) and at 1250 μg/ml the increase in mean mutant frequency was

only 34 mutants per 10^6 viable cells, compared to the concurrent negative control, i.e. considerably below the GEF. Furthermore, the increase in mutant frequency at 1400 µg/mL was associated with a decrease in pH of 1.85 and an increase in osmolality of 61 mOsm/kg, compared to concurrent negative control values, therefore the observation is considered to be of highly questionable biological relevance.

In Experiment 1 in the presence of S-9, an increase in mean mutant frequency of 152 (thus exceeding the GEF) was observed only at 1500 µg/ml (giving 3% RTG). At the lower concentration of 1250 µg/ml (giving 37% RTG), the increase in mean mutant frequency was only 4 mutants per 10^6 viable cells, compared to the concurrent negative control, i.e. between replicate cultures was observed at 1500 µg/ml, in addition to a decrease in pH of >1 unit and an increase in osmolality of >50 mOsm/kg, therefore the increase in mutant frequency at this concentration was not considered biologically relevant.

In the absence and presence of S-9 in Experiment 2, the mutant frequencies of the concentrations plated were all less than the sum of the mean control mutant frequency plus the GEF, indicating a negative result, but statistically significant linear trends were observed.

It is concluded that parapyruvate/lactone in HCl solution showed evidence of mutagenic activity when tested in the absence of S-9 in this test system. However, following 3 h treatment the increase was associated only with extreme toxicity (<10% RTG), a decrease in pH of >1 unit and an increase in osmolality of >50 mOsm/kg. Furthermore, following 24 h treatments in the absence of S-9, no marked increases in mutant frequency (exceeding the GEF) were observed at any concentration analyzed, although there was a statistically significant linear trend. Overall, these observations were considered of highly questionable biological relevance.

In the presence of S-9, parapyruvate/lactone in HCl solution did not show reproducible evidence of mutagenic activity when tested up to highly toxic concentrations.

5.3.4.5.3 Covance study number: 2789/10 (GE study number: B101064) Parapyruvate/lactone in TRIS solution: Induction of micronuclei in the bone marrow of treated rats (GLP)

Parapyruvate/lactone in TRIS solution was tested for its ability to induce micronuclei (MN) in the polychromatic erythrocytes (PCE) of the bone marrow of treated rats.

Parapyruvate/lactone in TRIS solution was formulated according to the user instruction, assuming the stock solution comprised concentrations of 10 mg/ml for each of the main components, parapyruvate and lactone. However, the certificate of analysis showed that the stock solution contained 8.6 mg/ml parapyruvate and 8.19 mg/ml lactone. The difference between the target assay value and the observed assay value was attributed to increased water content in the dry dispensed substance. Accordingly, the actual concentrations for parapyruvate and lactone were approximately 14-18% less than expected.

In an initial toxicity Range-Finder Experiment, out-bred male and female Han Wistar rats were dosed once with the test article at a maximum permissible dose volume of 20 ml/kg, designed to provide a dose of 200 mg parapyruvate/kg and 200 mg lactone/kg, via intravenous infusion at a rate of 1.2 ml/min. During a two-day post-dose observation period, clinical signs of piloerection were noted.

From these data, the nominal dose of 200 mg/kg was considered a suitable maximum dose for the Micronucleus Experiment. Two lower nominal doses of 60 and 20 mg/kg were also administered. No substantial difference was observed between males and females in the Range-Finder, therefore male animals only were used in the Micronucleus Experiment.

Groups of six male rats were treated once with the vehicle or parapyruvate/lactone in TRIS solution using the same dosing regimen as the Range-Finder Experiment at dose volumes of 2, 6 and 20 ml/kg to provide the doses stated above. A group of six male rats were treated once via oral gavage with the required positive control (cyclophosphamide 20 mg/kg) at a dose volume of 10 ml/kg.

No clinical signs were observed in the Micronucleus Experiment. Bone marrow smears were prepared from sacrificed animals approximately 24 and 48 h post-treatment.

Rats treated with parapyruvate/lactone in TRIS solution at all doses, and both sampling time points exhibited group mean % PCE that were similar to the values for the vehicle control group, and which were comparable with historical control data, thus confirming there was no evidence of test article related bone marrow toxicity.

Rats treated with parapyruvate/lactone in TRIS solution at all doses exhibited individual MN PCE frequencies that were generally similar to the values for the concurrent vehicle control group and which also fell within the laboratory's historical distribution data. There were no instances of statistically significant increases in micronucleus frequency for any of the groups receiving the test article.

It is concluded that parapyruvate/lactone in TRIS solution did not induce micronuclei in the polychromatic erythrocytes of the bone marrow of male rats treated up to the nominal dose of 200 mg/kg (the maximum practicable dose) following both 24 and 48 h sampling under the conditions of this assay.

5.3.5 Carcinogenicity

No studies have been performed to date.

5.3.6 Reproductive and Developmental Toxicity

No studies have been performed to date.

5.3.7 Local Tolerance

5.3.7.1. TNO study number: 5612/08 (GE study number: B101013): Local Tolerance Study with AH111501 dissolved in ^{12}C AH110896 in Rabbits (GLP)

The test item containing 44 mg/ml pyruvate and 0.85 mg/ml AH111501 in TRIS/EDTA dissolution medium was administered via the following routes: intra-arterial (0.5 ml per site in two males and two females), intramuscular (0.5 ml per site in two males and two females), subcutaneous and paravenous (0.2 ml per site in two males and two females) injection in albino rabbits. In addition, the acute dermal and eye irritating properties were examined, respectively, after one single dermal

application to the skin of three albino rabbits (0.5 ml per site), and one single application to the eye of three albino rabbits (0.1 ml per site).

The test item did not induce substance-related local effects in the rabbits by the dermal, ocular, intra-arterial and intramuscular route. Injection in the subcutis induced a minimal local inflammatory response in one out of four cases, consisting of scattered single-cell necrosis and infiltration with granulocytes, macrophages and monocytes. Injection in the paravenous area induced a moderate local inflammatory response in two out of four cases, generally consisting of hemorrhages, scattered single-cell necrosis and infiltration with granulocytes, macrophages and monocytes.

It was concluded that the test item containing pyruvate (44 mg/ml) and AH111501 (0.85 mg/ml) did not induce signs of local intolerance in rabbits by the dermal, ocular, intra-arterial, or the intramuscular route. Injection in the subcutis and in the paravenous area induced, respectively, a minimal and a moderate local inflammatory response

5.3.8 Other Toxicity Studies

No specific studies have been performed to date.

5.3.9 Discussion and Conclusions

Toxicology studies have included expanded acute toxicity studies in the rat and the dog, an extensive genetic toxicology package, as well as local tolerance studies, all conducted to GLP.

Two expanded acute toxicity studies were conducted in the rat with different concentrations of the pyruvate test article, one comprising 500 mM (44.6 mg/ml) [1-^{13}C]pyruvate plus 0.85 mg/ml AH111501, and the other 250 mM (22.3 mg/ml) [1-^{13}C]pyruvate plus 0.43 mg/ml AH111501. No treatment-related adverse effects were observed in either study; therefore, the top dose achieved, 1784 and 892 mg/kg [1-^{13}C]pyruvate, respectively, was the NOAEL in each of the studies. Similarly, the top dose of the excipient AH111501 was 34 mg/kg and 17 mg/kg, respectively, and was also regarded as the NOAEL in each of the studies. The top dose in these studies was limited by the maximum dose volume of 20 ml/kg on each of the two dosing occasions.

A single-dose expanded acute toxicity study was performed in the dog with the more concentrated pyruvate test article comprising of 500 mM [1-^{13}C]pyruvate plus 0.85 mg/ml AH111501. In common with the rat studies, there were no treatment-related adverse findings, and the top doses of 446 mg/kg [1-^{13}C]pyruvate and 8.5 mg/kg AH111501 were regarded as the respective NOAEL values. The top dose in this study was limited by the maximum dose volume for dogs of 5 ml/kg on each of the two dosing occasions.

The NOAEL, when expressed as the human equivalent doses (HED), is calculated to be 288 mg/kg for the acute dose rat study in which the highest dose of [1-^{13}C]pyruvate was administered, and for the acute dose dog study, the HED is 248 mg/kg. Similarly, the NOAEL for AH111501 expressed as the HED is 5.5 mg/kg and 4.7 mg/kg when based on data from the high dose rat study and the dog study, respectively.

The acute toxicity studies in rats to examine the effects of the potential impurities AH112623,

AH112615 and AH113462 resulted in no overt adverse effects at doses up to 1216, 1400 and 164 mg/kg, respectively. Accordingly, these doses were regarded as the NOAEL for the respective impurities, and these levels give HED values of 196 mg/kg for parapyruvate, 226 mg/kg for AH112615 and 26.4 mg/kg for AH113462.

The test article for the Phase 1/2a clinical study will comprise 250 mM [1-^{13}C]pyruvate (22 mg/ml) and 4.6 µg/ml AH111501/ml, given in ascending doses from 0.14 to 0.43 ml/kg bw. This gives a dose range of 3.1 to 9.6 mg/kg for [1-^{13}C]pyruvate and 0.64 to 1.97 µg/kg for AH111501. For the high-concentration test article (500 mM [1-^{13}C]pyruvate) and taking the more conservative NOAEL values converted to HED from the dog study, the safety margins for [1-^{13}C]pyruvate are from 79 to 26 and those for AH111501 are from 7362 to 2397 for the doses of 0.14 to 0.43 ml/kg bw, respectively. Similar calculations using data from the rat study with the 250 mM [1-^{13}C]pyruvate test article gives safety margins of 92 to 30 for pyruvate and 8549 to 2783 for AH111501.

Local tolerance studies in rabbits demonstrated a minimal and a moderate risk of local inflammatory response in case of misinjection in subcutis and in the paravenous area, respectively. The minimal inflammatory response was observed in one of four rabbits following injection in the subcutis, and the moderate inflammatory response was observed in two of four rabbits following injection in the paravenous area.

A number of studies have been performed to evaluate the genotoxic potential of the [1-^{13}C]pyruvate test article, the excipient AH111501 alone, and also the potential impurities AH112623, AH112615 and AH113462. In the conventional *in vitro* Ames test with the standard five strains of *Salmonella typhimurium*, there were no findings that were regarded as positive with any of these test items. However, in the MLA test using mouse lymphoma L5178 cells, which are sensitive to chemicals that induce gene mutations at the *tk*-locus, several positive findings were reported.

The pyruvate test article has been investigated in two separate MLA studies. In the first study, there was an increase in mutation frequency with the pyruvate test article, but also with the vehicle and TRIS/EDTA dissolution medium controls. In the cells treated with the pyruvate test article, this was only seen after exposure for 24 h in the absence of an S9 metabolizing system. In the second MLA study, there was a dose-related increase in mutation frequency after treatment incubation for 3 h with and without S-9, and after treatment incubation for 24 h without S-9. In both studies, there were increases in both large and small colonies, with greater increases in the proportion of small colonies. The positive effects were seen at high concentrations only, and these were shown to frequently be the cause of elevated osmolalities. The potential impurities AH112623, AH112615 and AH113462, as well as the novel excipient AH111501 have also been evaluated in the MLA test. AH112623 was found to be positive but only for concentrations for which the osmolality was substantially increased. AH113462 showed evidence of mutagenic activity but this was only associated with extreme toxicity, considerable changes in pH and osmolality, and these observations were considered of highly questionable biological relevance. AH111501 were positive but the effect was weak, whereas AH112615, was negative.

There is some uncertainty over the significance of these findings in the MLA test, as it is known to be prone to high incidences of false-positive results. In addition, it is unclear whether the positive results are due to the intrinsic properties of one or several of the constituents, or to some other factors such as electrolyte imbalance, pH changes or osmolality, the latter shown in several studies to be high due to the high concentrations of test item used, particularly at the levels associated with positive findings. If these physicochemical changes are associated with the

increased mutation frequency in the MLA assay, they are not relevant for a test article injected *in vivo* where the buffering capacity and rapid dilution in the bloodstream will rapidly normalize the injection solution to more physiological values.

To investigate the potential genotoxic risk of the test article, two mammalian *in vivo* assays were performed in rats. The micronucleus assay was done to test for the induction of erythrocyte micronuclei in the bone marrow. In this test, rats were given a maximum dose of 892 mg [1-^{13}C]pyruvate/kg bw/day and 17 mg AH111501/kg bw/day. The results of this study show that there were no increases in the incidences of micronuclei. The Comet assay was done to investigate induction of DNA strand breaks and/or alkali labile sites in blood. The maximum dose administered was 1784 mg [1-^{13}C]pyruvate/kg bw/day and 34 mg AH111501/kg bw/day on 2 consecutive days. Under the conditions of the assay, the test article did not induce DNA damage in the blood of rats. Based on the results of the two assays, the risk of this pyruvate test article being genotoxic *in vivo* is considered to be very low. The micronucleus assay was also done for the potential impurity AH113462 to test for the induction of erythrocyte micronuclei in the bone marrow. In this test, rats were given a single intravenous dose of 164 mg AH113462/kg bw (and 172 mg AH112623/kg bw). It was concluded that this, the maximum practicable dose, did not induce any effects at the 24 h and 48 h sampling.

The genetic toxicology studies can be summarized as follows: The data show that one of the two *in vitro* tests (Ames test) is negative for all of the test items that have been tested, i.e., the pyruvate test article, each of the three potential impurities, AH112623, AH112615 and AH113462, and the novel excipient, AH111501. The positive findings in the MLA test with the pyruvate test article and AH112623, and the weakly positive findings with AH111501 and AH113462, indicate that there is a potential hazard, although this is mitigated by the negative result in the standard *in vivo* risk assessment micronucleus study and the novel Comet assay with the pyruvate test article, and the micronucleus study for AH113462 (and AH112623).

In terms of human risk assessment, the weight of evidence from the toxicology studies, i.e., acute toxicity, local tolerance and genetic toxicology studies, indicates that the risk of the pyruvate test article causing toxicity is acceptably low.

5.4 Tabulated Summary of Non-clinical Studies

Study Title	Study No.	Results Summary
Primary Pharmacodynamic Studies		
Metabolic Imaging with hyperpolarized [1-^{13}C]-Pyruvate in Prostate of Healthy Dogs Using a Second-Generation Endorectal Coil and Reduced Dose	B101059	The injection rate was scaled to cardiac output to make it comparable to the clinical injection rate of 5 ml/s. The doses of 0.18 and 0.36 ml/kg were administered manually due to the low dose volume (2.0-4.7 ml). The dose of 1.4 ml/kg bw was administered by power injector. The study demonstrated pyruvate and lactate signal-to-noise ratios of ~20:1 for both the 0.18 and 0.36 ml/kg bw doses. The choice of dose appears to be limited by tissue contrast-to-noise and the highest acceptable dose from a safety perspective is recommended for clinical studies. Based on the results it is clear that the pulse sequence must be tailored to suit the human biology, and this can only be accomplished in clinical studies.
Safety Pharmacology Studies		
Na-Pyruvate and AH111501: Effects on HERG-1 tail currents recorded from stably transfected HEK 293 cells.	RCC 858776 (B101021)	Neither AH111501 nor Na-pyruvate affected the amplitude of the hERG-1 tail current at the tested concentrations. Exposure was demonstrated for Na-pyruvate at a concentration of 4.0 mg/ml.
AH111501 dissolved in ^{12}C-pyruvate: Modified Irwin screen test in the rat.	RCC 855869 (B101011)	Intravenous administration of test item containing AH111501 and pyruvate up to dose volumes of 5 ml/kg bw had no significant effects on the behavior of male Wistar rats when evaluated in a modified Irwin screen test.

Study Title	Study No.	Results Summary
Cardiovascular assessment in anaesthetised dogs of pyruvate administered at high injection rates.	B101026	One female dog died after receiving the first dose of 126 mg/kg bw pyruvate. This animal had received ~4.5 times as much pentobarbital and more than 1.6 times as much fentanyl per hour compared to the average given to the other dogs. The heavy dose of anesthetic was thought to have abolished all cardiovascular compensatory mechanisms. Pyruvate caused an acute, short-lasting, dose-dependent decrease in arterial blood pressure, immediately followed by a compensatory increase in heart rate and femoral arterial flow. Pulmonary arterial pressure [PAP] gradually increased with peak effect at 2-3 min. The increase in PAP was either a passive effect, caused by an increase in cardiac output, or by an increase in pulmonary resistance. A short-lasting, dose-dependent, prolongation of the QT/QTcV-interval was observed. No evidence of treatment-related arrhythmias was recorded. Administration of TRIS/EDTA vehicle control caused an acute short-lasting decrease in blood pressure. Osmotically-matched glucose solution caused effects like administration of pyruvate. A reduction in injection rate appeared to diminish the effects on systemic arterial pressure and heart rate and to a lesser degree the effect on pulmonary arterial pressure. Pyruvate was rapidly metabolized to lactate following intravenous injection, with high concentrations observed as early as 1 min post dosing. Tmax was in the range of 1 to 10 min. The pharmacokinetics of pyruvate and lactate were calculated using noncompartmental analysis. The blood concentrations of pyruvate and lactate were corrected for pre-dose levels before pharmacokinetic analysis. There was no statistically significant difference in Cmax or AUCtot between filtered and unfiltered test substance in any of the dose groups, suggesting that the presence of AH111501 in the test article did not influence the blood concentration of pyruvate. Pyruvate was eliminated from blood with an average elimination half-life of 18±11 min. Dose-normalized pyruvate data indicated that Cmax deviated negatively from proportionality in the 1.4 ml/kg bw dose group. AUCtot increased proportionally with dose. Dose-normalized lactate data indicated that Cmax and AUCtot deviated positively from proportionality in the 1.4 ml/kg bw dose group. Due to the individual variation in data, the biological significance of these findings is not known.

Study Title	Study No.	Results Summary
Mechanistic study of effects of 500 mM pyruvate on the cardiovascular system in anaesthetised male dogs.	B101031	A drop in arterial blood pressure was observed after pyruvate injections. Data from administration of hypertonic and isotonic test item indicate that the drop was caused by peripheral vasodilation and that the vasodilation was caused in part by the osmolarity of the test article and in part by a metabolite of pyruvate, probably lactic acid. Pyruvate caused no negative effect on the heart's pumping activity. There was no direct effect of pyruvate on the pulmonary circulation, the observed increase in pulmonary arterial pressure was a reflex of an increased cardiac output. Pyruvate was accompanied by a bi-phasic response in cardiac workload, the maximum increase (40% for 2 ml/kg) was of the same magnitude as variations seen during everyday activities (meals, postural changes, walking). A small increase (2 mmHg) in pulmonary venous pressure was observed after 2.0 ml/kg bw pyruvate injections. This was too small to have any effects on pulmonary tissue fluid balance and will not induce lung edema.
Effects of rapid intravenous administration of a mixture of natural abundant pyruvic acid and 15 mM AH111501 dissolved in TRIS/EDTA dissolution medium on the cardiovascular function in the telemetered dog.	Porsolt 06.075/5 (B101036)	Administration of test item and filtered test item caused similar effects. In doses of 4.3 and 5.7 ml/kg the test articles caused a non-significant, transient and slight increase in arterial blood pressure. At 5.7 ml/kg, the increase was preceded by a non-significant, weak and transient reduction in arterial blood pressure immediately after administration. Heart rate was increased compared to baseline by the test articles when administered in doses of 4.3 and 5.7 ml/kg. The increases for 5.7 ml/kg of test item and 4.3 ml/kg of filtered test article were also significant when compared to saline. No substantial effect on the PR- or QT-intervals was observed after administration of the pyruvate test articles. The test articles did not significantly modify the QTc interval (Fridericia's and van de Water's formulae) as compared with physiological saline. When compared with baseline, a significant lengthening was observed (Fridericia's) at 4.3 and 5.7 ml/kg and for the test article and at 4.3 ml/kg for the filtered test article. This lengthening was mainly ascribed to the delay in the QT-interval adaptation to the sudden variation of heart rate.

Study Title	Study No.	Results Summary
Safety pharmacology study of hemodynamic effects of a mixture of natural abundant pyruvic acid and 15 mM AH111501 dissolved in TRIS/EDTA dissolution medium after intravenous administration in the anesthetized dog.	Porsolt 06.076/6 (B101037)	Pyruvate caused an acute dose dependent peripheral vasodilation triggering a reduction in systemic arterial blood pressure, reflex tachycardia and an increase in cardiac output. dP/dt_{max} was decreased at the same time as the arterial blood pressure was reduced. Left cardiac work was unchanged for about the first minute after dosing with test item but increased acutely after injection of filtered test item. Left-ventricular end diastolic pressure and pulmonary artery blood pressure increased. After the acute phase, systemic arterial blood pressure, dP/dt_{max}, left cardiac work and stroke volume was increased. The maintained increase in cardiac output was due to the increase in myocardial contractility together with remaining tachycardia. Cerebral blood flow increased moderately. The PR interval was transiently shortened, at the same time as heart rate peaked. The QTc (van de Water's formula) interval was transiently lengthened after administration.
Evaluation of a mixture of natural abundance pyruvic acid and AH111501 dissolved in TRIS/EDTA dissolution medium on EEG and behaviour in the conscious dog.	Porsolt 06.210/5 (B101039)	Rapid bolus injections of the test item did not provoke pathological clinical symptoms or pathological EEG activity. The occasional symptoms observed were of a non-specific nature, transient or appeared before and after substance administrations and could not be attributed to either the substance or the composition of the vehicle. Convulsions and other pathologic signs were not observed following administration of test item in any of the 3 dogs tested. However, convulsions were observed following infusion of positive control in all 3 dogs confirming the sensitivity of the model.
Safety pharmacology study of hemodynamic effects after intravenous administration in the anesthetized dog of a mixture of natural abundant pyruvic acid and 15 mM AH111501 dissolved in TRIS/EDTA dissolution medium with AH111501 subsequently removed by solid phase extraction	Porsolt 06.434/4 (B101049)	As seen in study 06.076/6, rapid bolus injections of the pyruvate test article caused an acute dose dependent peripheral vasodilation, triggering dose dependent acute decreases in systemic arterial blood pressure and compensatory increases in heart rate. An acute onset and prolonged increase in cardiac output was observed. Left cardiac work increased immediately after administration. The initial increase (at 30 s) for this parameter was like the increase observed for the respective saline controls. When considering the measured effects as change from baseline, peak effects compared to the appropriate saline control and AUC for the duration of the peak (where available) compared to the appropriate saline control the effect level for this study was 2.1 ml/kg bw. The dose of 1.4 ml/kg bw caused only minor, though statistically significant, peak effects in systemic arterial blood pressure, left-ventricular end-diastolic pressure dP/dt_{max}, heart rate and QTcV when compared to the saline volume control. The effects were considered to be within normal physiological variation and the dose was considered to be No-Observed-Adverse-Effect–Level (NOAEL) for the study.

Study Title	Study No.	Results Summary
Pharmacokinetic Studies		
Study of Distribution and Excretion of [1-14C]Pyruvate in Male Sprague-Dawley Rat	B101003	After IV injection of sodium [1-^{14}C] pyruvate, radioactivity was rapidly distributed throughout the body. A dose of 56 mg sodium pyruvate/kg (including both [1-^{13}C] and [1-^{14}C] pyruvate) resulted in a blood concentration of radioactivity corresponding to 8.9% id (85.5 µg pyruvate equivalents/g blood) at 30 s post dosing. The initial volume of distribution was estimated to 677 ml/kg, indicating distribution of pyruvate to a volume comparable to total body water. The highest concentration of radioactivity 30 s post dosing was found in the pancreas. Relatively high concentrations were also found in the blood, liver, adrenals, heart muscle and the small and large intestine wall. The lowest concentrations of radioactivity were found in white fat, testes, brain and spinal cord. The concentration of radioactivity in the prostate at 30 s post dosing was 1.87 kBq/g. This corresponds to a pyruvate equivalent concentration of 34.5 µg/g. The elimination rate in prostate during the first 2 min post dosing corresponded to a half-life of 1.5 min. The highest recovery of radioactivity was found in muscle and skin tissue. At 1 min post dosing, the recovery in muscle and skin was 55% and 19% id, respectively. At 60 min post dosing, the recovery in muscle and skin was 13% and 7% id, respectively. The recovery of radioactivity in whole body cryosections was rapidly decreasing. The autoradiograms indicate some elimination of radioactivity through the kidneys, but no visible amounts of urine were collected in the time-frame of the study. The major elimination route was through exhaled air. At 120 min post dosing, radioactivity corresponding to 63% id was recovered in exhaled air, because of the formation of $^{14}CO_2$.

Study Title	Study No.	Results Summary
Study of Distribution and Excretion of [1-14C] pyruvate in Male Sprague-Dawley Rats - follow-up of study B101003	B101018	At 15 min post dosing, the highest radioactivity concentrations were found in urinary bladder contents, bone mineral tissue and pancreas, whereas relatively high concentrations were found in liver, small and large intestine wall, bone marrow and salivary glands. The lowest radioactivity concentrations were found in brain, spinal cord, white fat and testes. The radioactivity concentrations decreased with time in all organs and tissues. At 24 h post dosing, the highest concentrations were found in urinary bladder contents, bone marrow, large intestine wall and skin. The lowest concentrations were found in brain, white fat and eye. The highest recovery was found in muscle, skin and bone tissue. At both 15 min and 24 h post dosing, these tissues accounted for approximately 70% of the whole-body recovery. The total recovery determined in whole body sections decreased from 55% of the injected dose (% id) at 15 min to 7.9% id at 24 h. The major elimination route was through exhaled air. At 24 h post dosing, radioactivity corresponding to 56% id was recovered in exhaled air, because of the formation of $^{14}CO_2$. The elimination rate decreased biexponentially with time. Only minor amounts of radioactivity were excreted in urine and faeces. 2.1% id was found in voided urine 24 h post dosing (including cage wash water). The amount of radioactivity in the urine bladder was included in the whole-body recovery calculations, assuming a residual urine volume of approximately 1 ml/205-gram body weight. The recovery in faeces was 0.26% id at 24 h post dosing. The total recovery was in the range of 53 to 67% id after 24 h. Despite the relatively low total recovery, the study is considered to give a satisfactory description of the biological fate of [1-^{14}C]pyruvate following intravenous injection in rats.
Toxicology Studies		
An expanded acute dose toxicity study with intravenously injected AH111501 dissolved in Na-pyruvate in male and female rats	B101020	There were no treatment-related adverse effects in male or female rats receiving acute intravenous injection of AH111501 in 500 mM pyruvate at 1784 mg pyruvate/kg. NOAEL 1784 mg/kg body weight.
Expanded acute dose intravenous toxicity study with AH111501 dissolved in ^{13}C-pyruvate in Beagle dogs	TNO 588 (B101017)	There were no treatment-related adverse effects in male or female Beagle dogs receiving acute intravenous injections of these dose levels of AH111501 dissolved in ^{13}C-pyruvate. NOAEL 446 mg/kg body weight. Toxicokinetic analysis showed rapid transformation of ^{13}C-pyruvate to ^{13}C-lactate, with a peak concentration of ^{13}C-lactate at 3 minutes post dosing. The elimination half-life of ^{13}C-pyruvate and ^{13}C-lactate was 6.4±0.9 and 6.4±0.6 minutes, respectively. No biologically significant differences in systemic exposure between male and female animals were observed.

Study Title	Study No.	Results Summary
An expanded acute dose toxicity study with intravenously injected AH111501 in 250 mM pyruvate dissolved in 100 mM TRIS/EDTA in Sprague Dawley rats	B101040	There were no treatment-related adverse effects in male or female rats receiving acute intravenous injection of 250 mM [1-^{13}C]pyruvate and 0.43 mg/ml AH111501 in 100 mM TRIS/EDTA dissolution medium at 892 mg [1-^{13}C]pyruvate/kg. NOAEL 892 mg/kg bw
An expanded acute dose toxicity study with intravenously injected parapyruvate in male and female rats	B101044	There were no treatment related adverse effects in male or female rats receiving acute intravenous injection of AH112623 at 1216 mg/kg. NOAEL 1216 mg/kg body weight.
An expanded acute dose toxicity study with Intravenously injected AH112615 (PA-TRIS) in male and female rats	B101046	There were no treatment related adverse effects in male or female rats receiving acute intravenous injection of AH112615 at 1400 mg/kg. NOAEL 1400 mg/kg body weight
Parapyruvate/Lactone in TRIS Solution: Expanded Acute Dose Toxicity Study in the rat	B101065	There were no treatment related adverse effects in male or female rats receiving single intravenous injection of AH112623/AH113462 at 100 or 200 mg/kg. NOAEL was 200 mg/kg body weight.
Local tolerance study with AH111501 dissolved in ^{12}C-pyruvate in Rabbits	TNO 5612/08 (B101013)	**Intra-arterial:** No signs of intolerance were observed at the injection site in any of the rabbits during the 14-days observation period. **Subcutaneous**: Injection in the subcutis induced a minimal local inflammatory response in one out of four cases, consisting of scattered single cell necrosis and infiltration with granulocytes, macrophages and monocytes. **Paravenous**: Injection in the paravenous area induced a moderate local inflammatory response in two out of four cases, generally consisting of hemorrhages, scattered single cell necrosis and infiltration with granulocytes, macrophages and monocytes. Intra-muscular: No signs of test item-related local adverse effects **Ocular**: No signs of eye irritation were observed. **Dermal application**: No signs of skin irritation
Micronucleus test in bone marrow cells of rats treated intravenously with AH111501 dissolved in ^{13}C pyruvate	TNO 5604/04 (B101016)	500 mM [1-^{13}C] pyruvate and 0.85 mg/ml AH111501 in 200 mM TRIS/EDTA dissolution medium did not produce chromosomal damage or damage to the mitotic spindle apparatus in the bone marrow indicator cells in male rats.
Mixture of 500 mM [1-^{13}C] pyruvic acid and AH111501 sodium salt dissolved in TRIS/EDTA dissolution medium: Detection of DNA damage in the blood of treated rats using the Comet assay	Covance 2789/6 (B101057)	500 mM [1-^{13}C] pyruvate and 0.85 mg/ml AH111501 in 200 mM TRIS/EDTA dissolution medium did not induce DNA damage in the blood of rats treated with up to 1784 mg pyruvate/kg/day on two consecutive days, when analysed 3 h after the last dose administration.

Study Title	Study No.	Results Summary
Parapyruvate/ lactone in TRIS solution: induction of micronuclei in the bone marrow of treated rats (GLP)	Covance 2789/10/(B101064)	Parapyruvate/lactone in TRIS solution did not induce micronuclei in the polychromatic erythrocytes of the bone marrow of male rats treated up to the dose of 200 mg/kg (the maximum practicable dose) following both 24 and 48-hour sampling.
Formulation containing 557 µM AH111501 and 500 mM ^{13}C Pyruvate (AH110896) in 200 mM TRIS: Reverse Mutation in five Histidine requiring strains of *Salmonella typhimurium*	Covance 2570/03 (B101043)	500 mM [1-^{13}C] pyruvate and 0.85 mg/ml AH111501 in 200 mM TRIS/EDTA dissolution medium did not induce mutation in five histidine requiring strains (TA98, TA100, TA1535, TA1537 and TA102) of *Salmonella typhimurium* when tested under the conditions of this study. These conditions included treatments at amounts up to 5352 µg/plate, in the absence and in the presence of a rat liver metabolic activation system (S- 9).
Formulation containing 557µM AH111501 and 500 mµ ^{13}C Pyruvate (AH110896 in 200 mM TRIS): Mutation at the Thymidine Kinase (*tk*) Locus of Mouse Lymphoma L5178Y Cells (MLA) using the Microtitre^{R} Fluctuation Technique	Covance 2570/04 (B101045)	500 mM [1-^{13}C] pyruvate and 0.85 mg/ml AH111501 in 200 mM TRIS/EDTA dissolution medium induced mutation at the tk locus of L5178Y mouse lymphoma cells when tested under the conditions employed in this study. These conditions included treatments up to toxic concentrations for 24 h in the absence of a rat liver metabolic activation system (S-9) and up to approximately 5000 µg/ml for 3 h in the absence and presence of S-9. The mutagenic activity following 3-hour treatments in the absence and presence of S-9 was observed at concentrations at which marked increases in osmolality were also seen.
Parapyruvate: Reverse mutation in five histidine requiring strains of *Salmonella typhimurium*	Covance 2570/05 (B101042)	AH112623 did not induce mutation in five histidine-requiring strains (TA98, TA100, TA1535, TA1537 and TA102) of *Salmonella typhimurium* when tested under the conditions of this study. These conditions included treatments at amounts up to 5000 µg/plate, in the absence and in the presence of a rat liver metabolic activation system (S-9).
Parapyruvate: Mutation at the Thymidine Kinase (*tk*) Locus of Mouse Lymphoma L5178Y Cells (MLA) using the Microtitre^{R} Fluctuation Technique	Covance 2570/06 (B101041)	AH112623 did not induce mutation at the *tk* locus of L5178Y mouse lymphoma cells when tested under the 3-hour treatment conditions employed in this study. These conditions included treatments up to 5000 µg/mL in two independent experiments, in the absence and presence of a rat liver metabolic activation system (S-9). Parapyruvate may induce mutation at the *tk* locus of L5178Y mouse lymphoma cells when tested under 24-hour treatment conditions in the absence of S-9 up to toxic concentrations. However, as increases in mutant frequency were only noted at a concentration where marked changes in osmolality occurred, it cannot be determined from this study data whether these results are due to true mutation induction or physiological effects of increases in osmolality.

Study Title	Study No.	Results Summary
AH112615: Reverse mutation in five histidine requiring strains of *Salmonella typhimurium*	Covance 2570/07 (B101047)	AH112615 did not induce mutation in five histidine-requiring strains (TA98, TA100, TA1535, TA1537 and TA102) of *Salmonella typhimurium* when tested under the conditions of this study. These conditions included treatments at amounts up to 5000 µg/plate, in the absence and in the presence of a rat liver metabolic activation system (S-9).
AH112615: Mutation at the Thymidine Kinase (*tk*) Locus of Mouse Lymphoma L5178Y Cells (MLA) using the Microtitre^{R} Fluctuation Technique	Covance 2570/08 (B101048)	AH112615 did not induce mutation at the tk locus of L5178Y mouse lymphoma cells when tested under the conditions employed in this study. These conditions included treatments up to 5000 µg/ml in two independent experiments in the absence and presence of a rat liver metabolic activation system (S-9).
AH111501: Reverse mutation in five histidine requiring strains of *Salmonella typhimurium*	Covance 2570/10 (B101055)	AH111501 did not induce mutation in five histidine-requiring strains (TA98, TA100, TA1535, TA1537 and TA102) of *Salmonella typhimurium* when tested under the conditions of this study. These conditions included treatment at amounts up to 5000 µg/plate in the absence and in the presence of a rat liver metabolic activation system (S-9).
AH111501: Mutation at the Thymidine Kinase (*tk*) Locus of Mouse Lymphoma L5178Y Cells (MLA) using the Microtitre^{R} Fluctuation Technique	Covance 2570/11 (B101056)	AH111501 did not induce mutation at the tk locus of L5178Y mouse lymphoma cells when tested under the 3-hour treatment conditions employed in this study. These conditions included treatments up to 5000 µg/ml in two independent experiments, in the absence and presence of a rat liver metabolic activation system (S-9). AH111501 induce mutation at the tk locus of L5178Y mouse lymphoma cells when tested under 24-hour treatment conditions in the absence of S-9, up to toxic concentrations. The effect was found to be very weak.
Parapyruvate/ lactone in HCl solution: Reverse mutation in five histidine requiring strains of *Salmonella typhimurium*	Covance 2789/8 (B101062)	Parapyruvate/lactone in HCl solution did not induce mutation in five histidine-requiring strains (TA98, TA100, TA1535, TA1537 and TA102) of *Salmonella typhimurium* when tested under the conditions of this study. These conditions included treatments at concentrations up to 5000 µg/plate, in the absence and in the presence of a rat liver metabolic activation system (S-9).

Study Title	Study No.	Results Summary
Parapyruvate/ lactone in HCl solution: Mutation at the thymidine kinase (*tk*) locus of mouse lymphoma L5178Y cells (MLA) using the Microtitre Fluctuation Technique	Covance 2789/9 (B101063)	Parapyruvate/lactone in HCl solution showed evidence of mutagenic activity when tested in the absence of S-9 in this test system. However, following 3-hour treatment, the increase was associated only with extreme toxicity (<10% RTG Relative Total Growth), a decrease in pH of >1 unit and an increase in osmolality of >50 mOsm/kg. Furthermore, following 24-hour treatments in the absence of S-9, no marked increases in mutant frequency (exceeding the GEF Global Evaluation Factor) were observed at any concentration analysed, although there was a statistically significant linear trend. Overall, these observations were considered of highly questionable biological relevance. In the presence of S-9, Parapyruvate/lactone in HCl solution did not show reproducible evidence of mutagenic activity when tested up to highly toxic concentrations.
Bacterial reverse mutation test with AH111501 dissolved in ^{13}C pyruvate	TNO V5605/15 (B101014)	The test item containing [1-^{13}C] pyruvate and AH111501 was not mutagenic in *Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100 and TA102 when tested under the conditions of this study. These conditions included treatment at amounts up to 5000 µg/plate in the absence and in the presence of a rat liver metabolic activation system (S-9).
Gene mutation test at the *t*k locus of L5178Y cells with AH111501 dissolved in ^{13}C pyruvate	TNO V5603/08 (B101015)	In the presence of S-9 the test item ([1-^{13}C] pyruvate formulation) was not mutagenic in this assay. In the absence of S-9, there was an increase in mutation frequency with the test item, but also with the vehicle and dissolution medium controls. In the cells treated with the test item this was only seen after exposure for 24 h in the absence of an S9 metabolizing system.

5.5 Summary of the literature on the effects of pyruvate in animals

Animal experiments have demonstrated various beneficial metabolic effects of sodium pyruvate (pyruvate) administration, particularly on the heart and during hemorrhagic shock. Pyruvate has also been shown to have neuroprotective effects, while other studies have indicated that high doses of hypertonic pyruvate solutions may increase total infarct area following permanent focal cerebral ischemia, and cause seizures in rodents. Pyruvate administration has also been shown to protect against toxic effects in liver.

General tolerability to pyruvate was demonstrated in a study by Thorn et al. [1996] where no toxic alterations (changes in blood and urine parameters, organ morphology) were found after intramuscular injections of pyruvate up to 600 mg/day for 30 days in mice and rats. Cardiovascular tolerance to intravenous infusion of 50% calcium- and 50% sodium pyruvate was demonstrated in anesthetized intact dogs [Yanos et al., 1994]. The animals were dosed at a rate starting at 0.25 mg/kg bw/min of pyruvate, and the infusion rate was doubled every 15 min until a value of 16 mg/kg bw/min was reached. Data were collected at baseline and after 15 min at each infusion level. The results showed increases in cardiac output, left ventricular contractility, and mixed venous oxygen saturation values, demonstrating that pyruvate is a positive inotrope with several important beneficial effects. Even though the mean serum calcium concentrations were increased, it was concluded that there were no significant negative effects related to pyruvate. All animals completed the protocol without significant hemodynamic instability or rhythm disturbance. There were no significant changes in hemoglobin, hematocrit, white blood cell count, or platelet count. Infusion of pyruvate was associated with a significant increase in arterial pyruvate concentrations relative to the controls. Heart rate and blood pressure were not affected. There was no significant effect of pyruvate on gas exchange, but a mild metabolic acidosis developed in the control animals, whereas this was absent in the pyruvate-treated animals. In an open-thorax model in anesthetized dogs, venous infusion of pyruvate (up to 32 mg/kg bw/min) was reported to reduce vascular resistance, increase heart rate and left ventricular stroke volume, but not to alter either left ventricle contractility or arterial blood pressure [Romand et al., 1995]. Adverse reactions to pyruvate administration have however also been published. In a study in mice, rapid IV injection (injection duration 5 s) of 10 ml/kg bw of hyperosmotic 1 and 2 M sodium pyruvate (1 and 2 g/kg bw, respectively) was found to cause symptoms ranging from hind limb paralysis, which disappeared within 10 s, to generalized clonic convulsions, respiratory arrest and death within a few seconds after the injection [Gonzales et al., 2005]. The reactions were not seen after injection of 0.25 and 0.5 M pyruvate (250 and 500 mg/kg bw, respectively).

Pyruvate has been reported to have beneficial effects on the heart. Thon et al. [1968] found that 5 mM pyruvate was efficiently used by the well-oxygenated in-situ perfused rabbit heart when added as the sole nutrient to phosphate-Ringer solution. Addition of 5 and up to 10 mM pyruvate to a Krebs-Henseleit buffer containing 5 mM glucose, 5 U/l insulin and 5 mM lactate was found to restore ventricular performance following reperfusion in the isolated working-heart model using hearts from guinea-pig [Bünger et al., 1989]. Infusion of 1 ml/min of 150 mM pyruvate in the left anterior descending artery in an open-thorax model in anesthetized dogs was found to increase regional contractility measured as systolic wall thickening in the normal and the stunned myocardium [Mentzer et al., 1989]. These findings were further explored by Zhou et al. [1995] in an open-thorax model in the anesthetized pig by cardiac microdialysis. The study demonstrated that intracoronary infusion of 1 ml/min of 150 mM pyruvate following myocardial stunning partially restored regional contractility without increasing regional oxygen

consumption. The infusion was found to increase the regional myocardial phosphorylation potential as measured by the ratio between creatine phosphate and creatine multiplied by inorganic phosphate. At 20 min after the end of pyruvate infusion these parameters had returned to pre-treatment values. Administration of pyruvate was found to significantly reduce infarct size following 60 min occlusion of the left anterior descending coronary artery in the anesthetized pig, compared to saline-treated animals [Kristo et al., 2004]. Pyruvate (300 mg/ml) was administered as an IV bolus of 100 mg/kg bw followed by intra- arterial infusion of 10 mg/kg/min, with treatment initiated 30 min before occlusion and continued during ischemia, or extended throughout 60 min after reperfusion. There were no differences in heart rate, mean arterial pressure or coronary blood flow between the groups before occlusion and for up to 3 h after reperfusion. Arterial blood pH, hematocrit, P_{CO_2}, P_{O_2}, Na^+, K^+, Ca^{2+} and HCO_3^- concentration were also similar across the groups. The effects of intravenous pyruvate have also been studied in an open-thorax model of cardiac arrest (5 min) and cardiopulmonary resuscitation in anesthetized dogs [Sharma et al. 2005]. Pyruvate was infusion at a rate of 0.125 mmol/kg/min during 5 min of resuscitation and for 25 min following recovery. The infusion increased the plasma concentration of pyruvate to approximately 3.6 mM and improved post-arrest myocardial contractility and carotid flow compared to saline-treated animals. At 2-3 h after resuscitation contractile function stabilized and ECG normalized in pyruvate-treated animals despite clearance of pyruvate. Sharma et al. [2007] further explored the effects of 30 min pyruvate pre-treatment (0.125 mmol/kg/min) on myocardial enzymes in the model. It was found that the pre-treatment alleviated inactivation of some myocardial metabolic enzymes (e.g., phosphofructokinase, citrate synthase, malate dehydrogenase) during arrest in part by enhancing pre-arrest activities. There may be an upper limit to the concentration range in which intravenous pyruvate has a therapeutic effect since Laughlin et al. [1993] reported that in a study on *in situ* canine hearts, the heart rate became erratic, and blood pressure fell when plasma pyruvate exceeded 9 mM. Further, Bünger et al. [1989] found pyruvate to be less effective in preserving reperfusion function in isolated working guinea-pig hearts at concentrations above 10 mM.

Pyruvate administration has been reported to protect against cerebral insults. Rats injected IP with sodium pyruvate (500-1000 mg/kg bw) within 1 h after 12 min of forebrain ischemia showed almost no neuronal death, and the mortality was markedly decreased in the pyruvate- protected groups (3.8 %) compared with the NaCl-injected group (58.1 %) [Lee et al., 2001]. The neuroprotective effect persisted for 30 days after the insult, without noticeable side-effects. The authors speculated that the effect could be due to protection against zinc neurotoxicity, which was stated as one of the likely key mechanisms of ischemic brain injury. When sodium pyruvate (100 mg/ml) was given IP to rats 30 min after induction of permanent cerebral ischemia, doses of 250 and 500 mg/kg bw appeared to increase survival and reduce neurological deficits when compared to rats treated with osmolarity-matched saline [González- Falcón et al., 2003]. The administration of pyruvate in these doses did not affect infarct volume. Pyruvate at a dose of 1000 mg/kg bw was, in the same study, found to increase neurological deficits and total infarct volume when administered 30 min before infarction. A study comparing IP and IV administration of sodium pyruvate following transient (1 h) and permanent focal cerebral ischemia in the rat demonstrated protective effects when pyruvate administration was started 30 min after reperfusion or occlusion [Yi et al. 2007]. Doses of 62.5-250 mg/kg bw reduced infarct volume in both stroke models compared to saline-treated animals when administered IP and against transient ischemia when administered IV. In the permanent occlusion model only doses of 125 and 250 mg/kg reduced the infarct volume.

Pyruvate at 500 mg/kg bw showed little protective effect. The neuroprotective effect was also

present when 125 mg/kg bw was administered IP at 1 h after permanent occlusion, and the effect lasted 14 days. Pyruvate-treated animals (125 mg/kg bw) had gained weight at 14 days after insult whereas saline treated animals failed to regain weight. Pyruvate administration furthermore preserved motor function compared to sham treated animals, with saline treated animals having significantly poorer motor function compared to sham. Pyruvate has also been shown to protect against neurological dysfunction following closed head injury in the rat [Zlotnik et al., 2007]. A 30-min IV infusion was started 75 min after injury, and doses of 0.1 to 1 mmol/kg bw rat were found to give an improvement in neurological severity score of approx. 70% at 48 h compared to 1 h after injury. Saline-treated animals were also found to recover, but the recovery was slower than and not as complete as for animals treated with 1 mmol/kg bw pyruvate. In a recent study of neurological recovery following 5 min of cardiopulmonary arrest and resuscitation in mongrel dogs, IV infusion of hyperosmotic sodium pyruvate (2 M) reduced oxidative stress during infusion, preserved neurological function at recovery Days 1 and 2 and prevented neuronal loss in the hippocampal CA1 subregion on recovery Day 3 compared to treatment with 2 M NaCl [Sharma et al., 2008]. Pyruvate was administered at a rate of 0.125 mmol/kg bw/min (13.75 mg/kg bw/min) during 5 min of open-chest cardiac compressions and for 55 min following recovery of spontaneous circulation, giving a minimum total dose of 7.5 mmol/kg bw (825 mg/kg bw). In a transgenic model of amyotrophic lateral sclerosis, pyruvate administered IP at a dose of 1000 mg/kg bw/week from 70 days of age was found not to affect disease onset but increase mean lifespan by 10.5% while improving motor function and preventing weight loss when compared to mice treated with saline. However, pyruvate has also been shown to have less positive effects on the CNS when administered in extremely high doses. Intraperitoneal injection of 10 ml/kg bw of hyperosmotic pyruvate (0.1-5.6 M) in 0.9% saline in mice at 30 min before infusion of the seizure-inducing agent pentylenetetrazol, was found to reduce the threshold for tail twitch and clonic seizures for the pyruvate test articles of 3.2 and 5.6 M (3.5 and 6.2 g/kg bw, respectively) [Gasior et al., 2007]. These pyruvate doses were also associated with motor impairment in a high proportion of the animals when tested by the inverted-screen test.

In a model of severe hemorrhagic shock in the pig, IV infusion of pyruvate was demonstrated to increase survival time and improve cerebral function [Morgan et al. 1999, Morgan et al. 2001]. In the study from 1999, 300 mg/ml pyruvate infusion at a rate of 1 g/kg bw/h for 1 h before, and 0.5 g/kg bw/h after start of hemorrhage increased the survival time significantly from approximately 83 min for saline-treated animals to approx. 151 min. In the study from 2001, a bolus of 100 mg/kg bw of 300 mg/ml was administered after 30 min of controlled bleeding followed by an infusion of 0.5 g/kg bw/h giving an arterial concentration of approx. 5 mM. The treatment prevented an 8-fold increase in cortical glutamate compared to saline, as measured by microdialysis in the frontal neocortex. In a third study in the model [Morgan et al. 2002] using the same pyruvate protocol as in the 2001 study, pyruvate was found to decrease indicators of hepatic apoptosis as measured by microdialysis and analysis of tissue samples. In a study of severe hemorrhagic shock in the rat, Ringer's solution containing 28 mM pyruvate instead of a racemic mixture of D and L lactate was found to decrease pulmonary apoptosis when infused IV during 45 min of resuscitation [Koustova et al. 2003].

The administered dose was equal to 3 times the lost blood volume. In the same model the group [Jaskille et al. 2006] found that this modified Ringer's solution also protected against an increase in markers of hepatic apoptosis when compared to conventional lactated Ringer's solution. In study of small-volume resuscitation following hemorrhage in sheep [Nascimento et al., 2007], 4 ml/kg bw pyruvate (150 mg/ml) followed by infusion of lactated Ringers's solution did not provide any hemodynamic or metabolic benefits over administration of 4 ml/kg bw saline (80 mg/ml).

Effects of oral administration of pyruvate on the liver have been studied in rats fed ethanol, or a diet high in fat and ethanol [Stanko et al., 1978]. Oral administration of pyruvate in combination with dihydroxyacetone (6.6 mg/100 g bw each) was found to protect against increases in the concentration of esterified fatty acid content in the liver following 10 days ethanol treatment though the compounds had no effect when administered on their own. Oral administration of pyruvate in combination with dihydroxyacetone and riboflavin reduced the increases in esterified fatty acid and triglycerides caused by 30 days of eating a diet high in fat and ethanol. Oral supplements of pyruvate and calcium pyruvate (10% of energy intake) were given to rats fed clofibrate was found to inhibit the increase in liver size by 70% and normalized the protein content of the liver [Stanko et al., 1995]. The results were taken to indicate that pyruvate can prevent peroxisomal proliferation and free radical formation, thereby inhibiting free radical-induced lipid peroxidation.

5.6 Summary of adverse events recorded in 13C Academic Network

A number of research sites and academic centers in Europe and the USA licensed the hyperpolarization technology from GE Healthcare. These sites were loosely organized in the 13C Academic Network. Included in this network is General Electric's Global Research Center. The centers were not under the control of GE Healthcare and were free to conduct their research in accordance with the guidance of their respective institutions. The only obligation they had to GE Healthcare is to report adverse findings occurring during their work with pyruvate/[1-^{13}C]pyruvate through 2009.

In an explorative animal study performed at General Electric's Global Research Center in Niskayuna, USA in 2007, 5 rats died following administration of pyruvate via a jugular vein-catheter. In the study a total of 26 rats anesthetized with ketamine and diazepam were dosed with test articles of 80 mM pyruvate with and without the addition of an explorative electron paramagnetic agent at a concentration of approximately 100 µM. All test articles also contained about 56 mM sodium hydroxide, 31 mM TRIS and 42 µM EDTA. These formulations of pyruvate are different to the development candidate for use in humans.

Injections were via the tail vein (n=7) at doses of 6.8 to 11.5 ml/kg and injection rates of 0.2 to 0.3 ml/s, via the jugular vein through a catheter (n=11) at doses of 5.7 to 7.7 ml/kg and injection rates of 0.15 to 0.33 ml/s, or via the carotid artery through a catheter (n=8) at doses of 4.5 to 11.0 ml/kg and injection rates of 0.1 to 0.2 ml/s. There was no physiological monitoring of the animals. Both the peripheral venous (tail vein) and the central arterial (carotid) injections with or without the electron paramagnetic agent were well tolerated, even though the actual injection rates were 2 to 6 times higher than the maximum recommended rate according to current good practice [Diehl et al., 2001]. Five animals received a central venous (jugular) injection of pyruvate containing the electron paramagnetic agent, and three of these rats died. During dosing these rats were in the MRI machine and it was thus not possible to monitor them closely. In order to study the time-course from start of injection to death, six rats were closely observed during jugular vein injection of pyruvate without the electron paramagnetic agent. In two of these animals, breathing stopped within 2 to 3 seconds of the start of the injection. Death was established based on lack of pulse and pale paws and eyes. No personnel with a toxicological background were consulted in advance of the study and, accordingly, the examination and collection of organs was not considered. No adverse findings were observed in the remaining four rats. Due to experimental procedures employed the findings were not considered relevant for safety considerations for the suggested clinical use.

Since this occurrence GE Healthcare pro-actively monitored all sites that licensed the hyperpolarizer technology through 2009. There were a total of 21 sites globally, all of which have provided reports highlighting the number of experiments performed and, where appropriate, adverse events. As of October 2009, 3620 injections of various exploratory formulations of pyruvate/[1-^{13}C]pyruvate have been given to 1805 animals (including 12 dogs, 21 pigs and 1772 rats and mice). Out of the 1772 rodents, 39 died following administration of pyruvate. There were no incidences of adverse events in dogs or pigs in the 13C Academic Network.

The deaths were discussed with the respective centers and were believed to be due to one, or in most cases a combination, of the following reasons:

a) Problematic animal handling (e.g., poorly controlled anesthesia, body temperature, etc.)
b) Inappropriate formulations, particularly low or high pH due to insufficient neutralization and/or buffering, and hypotonic test articles
c) Inappropriate injection procedures, such as a combination of extremely high injection rates and/or injection volumes as well as accidental injection of air
d) The use of animal models of disease where severely altered physiology may lead to a higher incidence of events

It was noted that the deaths commonly occur during the early-phase of research at the centers (first 6 months), when the centers are developing their formulation(s) of [1-^{13}C]pyruvate. In the initial research phase, issues also arise regarding inappropriate injection volumes, injection rates, and injection of air.

It is believed that none of the 39 incidences of death following injection of pyruvate in these animal studies give cause for concern with regard to clinical use of Hyperpolarized (13C) Pyruvate Injection.

NCI is not continuing active monitoring of academic non-clinical sites, since the initiation of clinical trials has established a safe dose and well controlled formulation for human studies. Results from published well-controlled studies will of course be reported.

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6. EFFECTS IN HUMANS

6.1 Beneficial effects of pyruvate infusions in patients

Pyruvate is reported to have several beneficial effects in humans when administered at supraphysiological concentrations. It appears to hold promise as a safe, effective ionotropic agent for treatment of hearts that have been reversibly injured (stunned) by ischemia/reperfusion stress. Pyruvate enhancement of cardiac function may result from one or more of the following mechanisms: increased cytosolic ATP phosphorylation potential and Gibbs free energy of ATP hydrolysis, enhanced sarcoplasmic reticular calcium ion uptake and release, decreased cytosolic inorganic phosphate concentration, oxyradical scavenging via direct neutralization of peroxides and/or enhancement of the intracellular glutathione/NADPH antioxidant system, and/or closure of mitochondrial permeability transition pores (for review, see Mallet [2002]). Pyruvate infusion in patients with alcoholic liver disease has also shown good therapeutic effectiveness, possibly due to the rapid gain of ATP and GTP, required to redress defective cells, and to the antioxidant action of pyruvate [Petkova et al., 2000].

6.2 Dosage and safety of pyruvate infusions in patients

Intravenous infusion of locally-prepared formulations of sodium pyruvate has been shown to be well tolerated by patients with chronic liver diseases at doses up to 86 g/day, and the beneficial effect of the treatment on the liver damage was also evident [Mateva et al., 1996; Thorn et al., 1996; Petkova et al., 2000].

In the study by Mateva et al. [1996], 10 volunteer patients with chronic liver diseases received a 10-day course of intravenous sodium pyruvate infusions with the following regimen: 54 g (150 mg/min, 6 h) on the first day, 72 g daily (150 mg/min, 8 h) on the 2nd to 5th days, and 72 or 86.4 g daily (150 or 180 mg/min, 8 h) on the 6th to 10th days. The first 3 infusions of sodium pyruvate produced significant decreases in the serum activities of alanine aminotransferase and aspartate aminotransferase, and 5 infusions resulted in decreases in alkaline phosphatase and gamma glutamyltransferase. Improvement of the severity of liver damage was apparent from CCLI (combined clinical and laboratory index), Child's classification and galactose half-life. A significant decrease in total and conjugated bilirubin was also established in patients with high initial values. Increased pulse rate and blood pressure, thirst, and headache appeared to be the most frequent side-effects of the treatment. Nausea, singultus, discomfort, and fever were rarely observed. No augmentation of the side-effects was found during the study. As expected, the pyruvate blood concentrations increased during the infusions, but the values returned to the reference ranges after the end of the treatment. No significant changes in blood glucose levels were found. Increased initial serum lactate values (mean 1.66 mmol) were found, but this result was, according to the authors, possibly conditioned by the severity of the disease and was not considered to represent a tendency toward increased serum lactate as such; one day after the last infusion the initial level was restored.

In the study by Thorn et al. [1996], sodium pyruvate was infused intravenously (54 or 64 g/day, 150 or 180 mg/min) for 3 days in 12 volunteers (1 healthy person and 11 persons with chronic liver disease). Examinations including liver enzymes, bilirubin, total protein, albumin, prothrombin time tests and specific tests (galactose tolerance), serum and urine contents of pyruvate, lactate, glucose, and sodium were measured daily before and after infusion. Side-effects were facial flush, thirst, headache, increased pulse rate and blood pressure, and dizziness. Abdominal pain, vomiting,

retrosternal oppression, muscle pain, back pain, mild peripheral edema were occasionally observed. Blood pyruvate and lactate increased significantly after each infusion and decreased to the reference values on the next morning. It is concluded that pyruvate infusions were well tolerated despite some side-effects, probably depending on the severity of the liver and other diseases.

In a pilot study to investigate the therapeutic effectiveness of sodium pyruvate infusions in patients with alcoholic liver disease, seven patients were infused intravenously for 15 days with 50 or 54 g/day of sodium pyruvate (100 mg/min) [Petkova et al., 2000]. Further, 8 patients were treated for 10 days with 54 g/day sodium pyruvate (150 mg/min in 6 h) on the 1st day, and 72 or 86.4 g/day (150 or 180 mg/min in 6 – 8 h) on the 2nd to 10th days. Three subjects dropped out of the study; 2 subjects because of phlebitis in the infusion vein (possibly as a result of the injection of sodium and the hyperosmolarity of the infusion solution) and 1 subject due to ascites and peripheral edema. During the study, 69% of the infusions were well tolerated and 26% very well tolerated. Increases in pulse rate and blood pressure within the reference ranges were noted but could be explained by the sodium- and volume-loading.

Serum activities of alanine aminotransferase, aspartate aminotransferase, and concentrations of total bilirubin significantly decreased after treatment with sodium pyruvate. Non-liver clinical tests including creatinine, hemoglobin, hematocrit, and blood count were also examined and no significant changes were found. No significant changes in serum and urine pyruvate, lactate or glucose levels were found during and after the treatment.

Cardiac hypertrophy, induced by chronic pressure or volume overload, is associated with abnormalities in energy metabolism as well as characteristic increases in muscle mass and alterations in the structure of the heart. Hypertrophied hearts display increased rates of glycolysis and overall glucose utilization, but rates of pyruvate oxidation do not rise in step with rates of pyruvate generation, resulting in increased level of pyruvate. However, the exact level of pyruvate in hypertrophied hearts is not reported [Leong et al., 2003].

In a study by Hermann et al. [1999], hemodynamic effects of intracoronary pyruvate in patients with dilated cardiomyopathy and heart failure were investigated. Pyruvate (150 mmol/l) was infused into the left main coronary artery at 370 ml/h for 15 min, then at 740 ml/h for 15 min. Pyruvate at supraphysiological concentrations increased stroke-volume index by 38% and decreased pulmonary capillary-wedge pressure by 36% in each patient. Heart rate decreased by 11%. Pyruvate significantly reduced mean artery pressure and pulmonary vascular resistance whereas mean aortic pressure and systemic resistance did not change. Femoral artery pyruvate concentrations were 0.04 mmol/l at baseline, and 0.13 mmol/l during pyruvate infusion. Lactate levels did not change during pyruvate infusion. No side-effects were observed. The effects of pyruvate may especially help failing hearts, on the assumption that energy starvation contributes to myocardial failure.

One fatal outcome in a 9-year-old boy with the diagnosis restrictive cardiomyopathy is reported [Matthys et al., 1991] after performance of the pyruvate loading test [Dijkstra et al., 1984; Van Erven et al., 1987a; Van Erven et al., 1987b; Van Erven et al., 1989; Trijbels et al., 1988], which is recommended in the diagnosis of mitochondrial enzyme deficiency. After overnight fasting a locally-prepared intravenous infusion of sodium pyruvate (0.5 g/kg bw) was given over 10 min. Since cardiac monitoring was not done, it is unclear whether the cause of death was asystole, ventricular fibrillation, or an abrupt fall in blood pressure. Lactate/pyruvate ratios were normal, but it is concluded that a sudden and severe increase in lactate concentration (10.4 mmol after 5 min and 9.7 mmol after 12 min) was probably not tolerated in this child with cardiomyopathy. Another patient with clinical and neuroradiological evidence of Leigh's syndrome had normal blood

concentrations of lactate and pyruvate but, after intravenous injection of pyruvate, values of lactate and pyruvate increased by 3 and 5 standard deviations, respectively, confirming the diagnostic usefulness of this test in screening for defects of pyruvate metabolism [Matthys et al., 1991]. The authors recommend that the test should not be done when cardiac function is decreased.

6.3 Clinical studies with Pyruvate Injection

Two clinical studies in healthy humans have been completed, one in 36 healthy male and female volunteers (GE-101-001) and one in 30 elderly male and female volunteers (GE-101-003). The studies were conducted in accordance with the Declaration of Helsinki and Good Clinical Practice (GCP).

6.3.1 A phase 1, placebo-controlled, randomized, ascending-dose study to assess the safety and tolerability of Pyruvate Injection in healthy male and female volunteers (GE-101-001)

The available preclinical data indicated sufficiently good safety margins to support testing the safety and tolerability of a 250 mM formulation of Pyruvate Injection containing 250 mM EPA (i.e., 22.0 mg/ml of pyruvate and 4.6 μg/ml of EPA) in healthy non-elderly human volunteers in this phase 1 dose-escalating study up to doses of 40 ml/70-kg volunteer (i.e., up to 12.6 mg/kg bw of pyruvate and 2.6 μg/kg bw of EPA). The data from the preclinical studies in conscious and anaesthetized dogs indicated that special attention should be paid to the possibility of mild cardiovascular effects occurring, similar to those reported when Pyruvate Injection was administered rapidly at large volumes to conscious dogs (increases in heart rate) and anaesthetized dogs (vasodilatation leading to small, short-lasting but significant reductions in blood pressure and compensatory increases in heart rate).

The primary objective of this trial was to determine the safety of Pyruvate Injection in a dose-ascending manner (0.07, 0.14, 0.28, 0.43, and 0.57 ml/kg bw) in healthy adult male and female volunteers, starting with the lowest of the 5 dose levels and using appropriate placebo-treated controls.

The study was conducted at the phase 1 unit: Clinical Research Services Turku, Turku, Finland. The subjects were recruited from a volunteer panel and by advertisement.

Thirty-six subjects were enrolled into 6 dose groups; the 6th dose group was a repeat of the 0.43 ml/kg dose (i.e., the 4th dose-group dose). Each group contained 6 subjects (4 received Pyruvate Injection and 2 received placebo). Doses from 0.07 to 0.57 ml/kg bw of a 250 mM formulation of Pyruvate Injection were intravenously injected at a rate of 5 ml/second. At the highest dose-level, subjects received 12.6 mg/kg bw of pyruvate and 2.6 μg/kg bw of EPA. The study commenced at the lowest dose of 0.07 ml/kg bw (5 ml for a 70-kg subject) and the highest dose was 0.57 ml/kg bw (40 ml for a 70-kg subject). The choice of doses was initially guided by limitations in the maximum volume (~50 ml) of hyperpolarized Pyruvate Injection that can be produced for future imaging studies using the special equipment built for this purpose.

The subjects randomized to receive placebo were given an intravenous injection of Sodium Chloride Solution for Infusion (0.9% w/v) at an injection rate of 5 ml/second and at a volume that

corresponded with the volume of Pyruvate Injection administered in each dose group.

Blood samples for pharmacokinetic assessments were collected at baseline and at various time points up to 15 minutes after drug administration. Safety assessments including baseline signs and symptoms, adverse events (AEs), physical examinations, clinical laboratory tests (serum biochemistry, hematology, and urinalysis), vital signs (heart rate, systolic and diastolic blood pressures, respiration rate, body temperature, and oxygen saturation), and 12-lead ECGs were performed at various time points pre- and post-administration. In addition, the site of injection was monitored for evidence of local and regional reactions. Changes after administration of Pyruvate Injection were compared to changes after administration of placebo. Safety data up to and including the 2-day post-injection assessment were reviewed before proceeding to the next dose level. During the study, safety assessments were performed by an observer who was blinded to the nature (placebo or Pyruvate Injection) of the injection.

Increasing to the next dose level occurred after the safety data from the previous dose level had been thoroughly evaluated by the sponsor and the principal investigator and both agreed that it was safe to continue with the next dose. The study was temporarily stopped by the sponsor after completion of dosing for the 0.57 ml/kg dose group due to the occurrence of non-serious AEs in 2 subjects ('unresponsiveness' in subject 001-0026 and 'flushing' accompanied by changes in blood pressure and heart rate in subject 001-0030) that the principal investigator considered to be concerning and related to the administration of Pyruvate Injection. These events occurred in 2 of the 4 subjects in that dose group who received Pyruvate Injection. The sponsor decided not to proceed with the planned dose escalation to 0.71 ml/kg. The protocol was amended to repeat the 0.43 ml/kg dose group in place of the planned 0.71 ml/kg dose group to confirm that 0.43 ml/kg was as well tolerated in a second placebo-controlled cohort of 6 healthy volunteers as it was in the first one. Additional safety monitoring was considered but was deemed unnecessary.

Dose Groups of Investigational Medicinal Product (Pyruvate Injection and Placebo)

Dose Group	Pyruvate Injection (250 mM Pyruvate + 3 μM EPA)					Placebo[a] No. of Subjects
	Dose (ml/kg bw)	Dose (ml/70 kg)	Pyruvate Dose (mg/kg bw)	EPA Dose (μg/kg bw)	No. of Subject	
1	0.07	5	1.6	0.3	4	2
2	0.14	10	3.1	0.7	4	2
3	0.28	20	6.3	1.3	4	2
4	0.43	30	9.4	2.0	4	2
5	0.57	40	12.6	2.6	4	2
6	0.43	30	9.4	2.0	4	2

bw = body weight; w/v = weight in volume; EPA = electron paramagnetic agent.
[a]equivalent volume of saline: Sodium Chloride Solution for Infusion (0.9% w/v).

Demographics

Summary Statistics for Baseline Demographic Characteristics by Treatment Group

Variable		Dose Group					
		Pyruvate Injection					
		0.07 ml/kg N = 4	0.14 ml/kg N = 4	0.28 ml/kg N = 4	0.43 ml/kg N = 8	0.57 ml/kg N = 4	Placebo N = 12
Gender	Male	1 (25%)	1 (25%)	1 (25%)	3 (38%)	2 (50%)	4 (33%)
	Female	3 (75%)	3 (75%)	3 (75%)	5 (62%)	2 (50%)	8 (67%)
Race	Caucasian	4 (100%)	4 (100%)	4 (100%)	8 (100%)	4 (100%)	12 (100%)
Age (Years)	Mean	27.5	30.0	21.5	26.3	23.5	26.3
	SD	5.74	6.06	1.29	7.05	1.73	4.94
	Range (min-max)	21-35	22-36	20-23	19-39	22-26	22-37
Weigh t (kg)	Mean	73.5	65.3	61.0	68.0	67.8	70.6
	SD	20.87	6.85	4.24	13.77	13.50	12.03
	Range (min-max)	51-101	55-69	56-65	53-91	51-84	51-94
Height (cm)	Mean	169.3	169.5	170.5	172.9	173.3	169.8
	SD	11.0	7.42	8.43	6.88	9.00	8.77
	Range (min-max)	160-185	159-176	162-180	164-183	162-181	158-192
BMI (kg/m^2)	Mean	25.23	22.66	21.00	22.55	22.40	24.39
	SD	4.02	0.99	0.85	2.92	2.81	3.17
	Range (min-max)	19.9-29.5	21.8-23.9	19.8-21.7	19.2-27.2	19.4-25.9	20.4-30.3

BMI = Body mass index

Adverse events

Summary of All AEs by Treatment Group, Causality, Intensity, Seriousness, Withdrawals and Deaths

	0.07 ml/kg N = 4		0.14 ml/kg N = 4		0.28 ml/kg N = 4		0.43 ml/kg N = 8		0.57 ml/kg N = 4		Placebo N = 12	
	Subjects with an AE n (%)	AEs n	Subjects with an AE n (%)	AEs n	Subjects with an AE n (%)	AEs n	Subjects with an AE n (%)	AEs n	Subjects with an AE n (%)	AEs n	Subjects with an AE n (%)	AEs n
Any AE[a]	0 (0%)	0	3 (75%)	4	4 (100%)	5	5 (63%)	10	3 (75%)	16	3 (25%)	6
Causality[b]												
Related	0 (0%)	0	2 (50%)	3	3 (75%)	3	5 (63%)	10	3 (75%)	13	1 (8%)	1
Not related	0 (0%)	0	1 (25%)	1	1 (25%)	2	0 (0%)	0	3 (75%)	3	3 (25%)	5
Intensity[b]												
Mild	0 (0%)	0	3 (75%)	4	4 (100%)	5	5 (63%)	10	3 (75%)	12	3 (25%)	6
Moderate	0 (0%)	0	0 (0%)	0	0 (0%)	0	0 (0%)	0	2 (50%)	3	0 (0%)	0
Severe	0 (0%)	0	0 (0%)	0	0 (0%)	0	0 (0%)	0	1 (25%)	1	0 (0%)	0
Serious AEs	0 (0%)	0	0 (0%)	0	0 (0%)	0	0 (0%)	0	0 (0%)	0	0 (0%)	0
Withdrawals due to AE	0 (0%)	0	0 (0%)	0	0 (0%)	0	0 (0%)	0	0 (0%)	0	0 (0%)	0
Deaths	0 (0%)	0	0 (0%)	0	0 (0%)	0	0 (0%)	0	0 (0%)	0	0 (0%)	0

N = Total number of subjects in that treatment group.
[a] Subjects may have experienced more than 1 AE.
[b] Subjects are counted once for each subset in which they have recorded at least 1 AE of this causality or intensity.

Overall, 18 subjects (50%) experienced a total of 41 AEs. Of the 24 subjects given Pyruvate Injection, 15 subjects (63%) experienced 35 AEs. Of the 12 subjects given placebo, 3 subjects (25%) experienced 6 AEs. No SAEs or deaths were reported and no subjects were withdrawn due to AEs.

Similar numbers/percentages of AEs were reported across the Pyruvate Injection dose groups, with the exception of the 0.57 ml/kg dose group where a clearly increased number and percentage of total AEs was reported and some safety concerns were raised (see below).

Summary of AE Duration, Intensity, Relationship to Investigational Medicinal Product, and Outcome, by Treatment Group

Adverse Event	Subject Numbe	Serious (yes/no)	Time from Injection to Onset	Duration of AE	Intensity	Relationship to IMP	Outcome
0.14 ml/kg Pyruvate Injection							
Feeling hot	001-0007	No	0 min	2 min	Mild	Suspected	Resolved
Dry mouth	001-0007	No	0 min	2 min	Mild	Suspected	Resolved
Dizziness	001-0008	No	1 h 18 min	1 min	Mild	Not suspected	Resolved
	001-0009	No	0 min	2 min	Mild	Suspected	Resolved
0.28 ml/kg Pyruvate Injection							
Feeling hot	001-0013	No	0 min	5 s	Mild	Suspected	Resolved
	001-0014	No	0 min	<1 min	Mild	Suspected	Resolved
Flushing	001-0016	No	0 min	<1 min	Mild	Suspected	Resolved
Pharyngo-laryngeal pain	001-0017	No	6 h 25 min	30 min	Mild	Not suspected	Resolved
	001-0017	No	17 h 10 min	5 min	Mild	Not suspected	Resolved
0.43 ml/kg Pyruvate Injection							
Feeling hot	001-0023	No	15 s	30 s	Mild	Suspected	Resolved
Micturition urgency	001-0023	No	15 s	30 s	Mild	Suspected	Resolved
Dysgeusia	001-0023	No	15 s	30 s	Mild	Suspected	Resolved
Flushing	001-0023	No	0 min	2 min	Mild	Suspected	Resolved
	001-0033	No	5 s	55 s	Mild	Suspected	Resolved
	001-0034	No	10 s	1 min 40 s	Mild	Suspected	Resolved
	001-0035	No	10 s	50 s	Mild	Suspected	Resolved
Dizziness	001-0024	No	1 s	14 s	Mild	Suspected	Resolved
Parosmia	001-0033	No	5 s	15 s	Mild	Suspected	Resolved
	001-0035	No	10 s	4 s	Mild	Suspected	Resolved
0.57 ml/kg Pyruvate Injection							
Feeling abnormal	001-0026	No	15 s	1 min 45 s	Mild	Suspected	Resolved
Dizziness	001-0026	No	15 s	10 h 9 min 45 s	Mild	Suspected	Resolved
Unresponsive to stimuli	001-0026	No	15 s	30 s	Moderate	Suspected	Resolved
Migraine	001-0026	No	1 day 2 h 40 min	3 h	Mild	Not suspected	Resolved
Hypoesthesia	001-0028	No	5 min	5 min	Mild	Not suspected	Resolved
	001-0030	No	1 day 14 h	7 h	Mild	Not suspected	Resolved
Feeling hot	001-0028	No	0 min	2 min	Mild	Suspected	Resolved
Dysgeusia	001-0028	No	0 min	2 min	Mild	Suspected	Resolved
	001-0028	No	49 min	6 min	Mild	Suspected	Resolved

Summary of AE Duration, Intensity, Relationship to Investigational Medicinal Product, and Outcome, by Treatment Group

Adverse Event	Subject Numbe	Serious (yes/no)	Time from Injection to Onset	Duration of AE	Intensity	Relationship to IMP	Outcome
Fatigue	001-0028	No	6 h 20 min	8 h	Mild	Suspected	Resolved
Flushing	001-0030	No	0 min	1 min	Mild	Suspected	Resolved
	001-0030	No	20 s	30 s	Severe	Suspected	Resolved
Heart rate increased	001-0030	No	1 min	1 min	Moderate	Suspected	Resolved
	001-0030	No	6 min	4 min	Moderate	Suspected	Resolved
BP increased	001-0030	No	1 min	3 min	Mild	Suspected	Resolved
BP diastolic increased	001-0030	No	5 min	1 min	Mild	Suspected	Resolved
Placebo							
Catheter site pain	001-0011	No	40 min	10 min	Mild	Not suspected	Resolved
Dizziness	001-0011	No	12 h 10 min	30 min	Mild	Not suspected	Resolved
Injection site hematoma	001-0011	No	UNK	Ongoing	Mild	Not suspected	Not resolved
Pharyngo-laryngeal pain	001-0020	No	13 h 40 min	Ongoing	Mild	Not suspected	Not resolved
Oral herpes	001-0025	No	8 h 20 min	1 day 4 h	Mild	Not suspected	Resolved
Dysgeusia	001-0025	No	0 min	1 min	Mild	Suspected	Resolved

h = hour; min = minute; s = second; UNK = unknown

All 6 AEs in placebo subjects were mild in intensity. Thirty-one (89%) of the 35 AEs in the Pyruvate Injection subjects were mild in intensity and 3 (9%) were moderate in intensity.
There was 1 AE of severe intensity in the 0.57 ml/kg Pyruvate Injection dose group; subject 001-0030 experienced flushing which was suspected to be related to this investigational medical product (IMP).

A total of 30 AEs (73%) were suspected to be related to the IMP, 29 of 35 AEs (83%) in the Pyruvate Injection dose groups and 1 of 6 AEs (17%) in the placebo group. Since neither the subjects nor the safety observers were aware of which IMP had been given, this indicates that many of the events suspected of being related to Pyruvate Injection were truly related to the administration of this IMP. The most frequently reported individual AEs were flushing (6 subjects, 7 AEs), dizziness (5 subjects, 5 AEs), and feeling hot (5 subjects, 5 AEs). Flushing and feeling hot were reported only in subjects receiving Pyruvate injection and dizziness was mainly reported in subjects receiving Pyruvate Injection (4 out of 5 AEs) indicating that these events are linked to the administration of Pyruvate Injection.

Thirty-nine (95%) of the 41 AEs that were reported resolved before the final follow-up. The 2 AEs that did not resolve (injection site hematoma in subject 001-0011 and pharyngolaryngeal pain in subject 001-0020) were reported in the placebo group and were not suspected to be related to IMP.

Treatment was given for 3 AEs; subject 001-0026 (0.57 ml/kg Pyruvate Injection dose group) took Naproxen for migraine, subject 001-0011 (placebo group) took paracetamol for dizziness, and subject 001-0025 (placebo group) took acyclovir for oral herpes. None of these events was suspected to be related to the IMP given.

The investigator raised safety concerns over some of the AEs reported in the 0.57 ml/kg dose group:

Subject 001-0026 was a 51-kg, 21-year-old female with no history of allergy. When, soon after administration of Pyruvate Injection, the investigator asked if the subject was feeling OK, the subject was 'unresponsive' for approximately 30 seconds and could not answer the question. The subject recovered quickly and reported having experienced an 'odd feeling'. The investigator classified this as a non-serious AE that was related to Pyruvate Injection.

Subject 001-0030 was a 67-kg, 26-year-old female with no history of allergy. Soon after administration of Pyruvate Injection, the subject started gasping and her face became flushed. Afterwards she reported feeling a sensation of something touching her throat (not constriction). The subject's heart rate rose from a stable baseline value of 58 bpm to 93 bpm at 1 minute post-injection, declined to 61 bpm at 2 minutes post-injection, and rose again to 109 bpm at 6 minutes post-injection (data from ECG recordings). The subject's blood pressure also rose (particularly diastolic blood pressure) but not to an alarming level. When asked to grade the severity of her symptoms on a scale of 1-5, the subject graded the facial flushing as 1 and the throat feeling (coded as 'flushing, severe') as 5. The investigator classed these events as non-serious AEs that were related to Pyruvate Injection.

In addition, subject 001-0028 in this dose group experienced 4 AEs that were considered related to Pyruvate Injection, including feeling hot, dysgeusia and fatigue; however, these events did not raise any concern at the time.

In order to assess the AEs in the 0.57 ml/kg dose group that were classified as related to IMP and to facilitate a safety discussion, the blind was broken for 4 subjects prior to database lock. These were subject 001-0025 (placebo), and subjects 001-0026, 001-0028 and 001-0030 (all received Pyruvate Injection).

The study was temporarily halted after completion of the 0.57 ml/kg dose group. The sponsor decided not to proceed with the next planned dose level (0.71 ml/kg). The protocol was amended to repeat the 0.43 ml/kg dose group to confirm that this dose was as well tolerated in a second cohort of healthy volunteers as it was in the first one. The introduction of additional safety monitoring was considered but was not thought necessary in light of the comprehensive safety monitoring already being applied.

Safety Conclusions

- Among the 36 subjects enrolled into the study, 18 subjects (50%) experienced a total of 41 AEs. Of these, 15 out of 24 subjects (63%) in the Pyruvate Injection dose groups experienced 35 AEs and 3 out of 12 subjects (25%) in the placebo dose groups experienced 6 AEs. The majority of AEs were mild in intensity (37, 90%). No deaths, SAEs or withdrawals due to AEs occurred during the study.

- Similar numbers/percentages of AEs were reported across the Pyruvate Injection dose groups, with the exception of the 0.57 ml/kg dose group where a clearly increased number of AEs were reported and some safety concerns were raised. Although none of these events was considered to be an SAE, the sponsor decided not to proceed with the next planned dose level (0.71 ml/kg). The 0.43 ml/kg dose group was repeated and this dose was confirmed as well tolerated in a second cohort of healthy volunteers.

- A total of 30 of 41 AEs (73%) were suspected to be related to IMP; 29 of 35 AEs

(83%) in the Pyruvate Injection dose groups and 1 of 6 AEs (17%) in the placebo group. Since neither the subjects nor the safety observers were aware of which IMP had been given, this indicates that many of the events suspected of being related to Pyruvate Injection were truly related to the administration of this IMP.

- The most frequently reported individual AEs were flushing (6 subjects, 7 AEs), dizziness (5 subjects, 5 AEs), and feeling hot (5 subjects, 5 AEs). Flushing and feeling hot were reported only in subjects receiving Pyruvate Injection and dizziness was mainly reported in subjects receiving Pyruvate Injection (4 out of 5 AEs) indicating that these events are linked to the administration of Pyruvate Injection.

- Among the subjects in the 0.07 to 0.43 ml/kg dose groups, there was overall stability throughout the follow-up period for all parameters, including clinical laboratory, vital signs and ECG. No clinically important trends or safety signals were noted.

- In the 0.57 ml/kg dose group, subject 001-0030 showed changes in heart rate and blood pressure that were of concern to the investigator and were reported as AEs. In addition, this subject showed several increases in QTcB (Bazett's correction) interval >60 msec but only 1 increase in QTcF (Fridericia's correction) in this period. Since the subject's heart rate was ~90 or more when changes in QTc were noted, QTcF is the appropriate correction; therefore, these changes were not classified as being clinically significant.

- No SAEs occurred during the study and most of the non-serious AEs that occurred during the study were mild in intensity, short-lasting, and resolved spontaneously without any treatment or intervention. Throughout the study, serum biochemistry, hematology, urinalysis, vital signs and ECG variables showed overall stability and no dose-related tendencies (with the exception of the changes in heart rate and blood pressure that were experienced by subject 001-0030 in the 0.57 ml/kg dose group and reported as AEs). No other clinically important trends or safety signals were noted.

Overall safety conclusion

In conclusion, doses of Pyruvate Injection up to 0.43 ml/kg were well tolerated in young healthy male and female volunteers. The safety and tolerability of Pyruvate Injection, up to 0.43 ml/kg, should now be assessed further in elderly volunteers (i.e., subjects ≥60 years of age), to confirm an acceptable safety profile in subjects in whom compensatory baroreflexes are expected to be compromised as a result of normal ageing. This assessment is of particular interest as the target population for this product – patients with prostate cancer – will tend to be elderly.

Throughout the study, serum biochemistry, hematology, urinalysis, vital signs and ECG variables showed overall stability and no dose-related tendencies. No clinically important trends or safety signals were noted.

While it is apparent that the percentage of AEs attributed to IMP is higher in subjects given Pyruvate Injection than those given placebo, most of these events (e.g., flushing, dysgeusia, and dizziness) were mild, short-lasting symptomatic reactions that are commonly reported after intravenous injection of 10 ml or more of other types of contrast agents, e.g., those used for X-ray and MRI examinations. Therefore, this difference does not raise any concerns.

6.3.2 A phase 1, placebo-controlled, randomized, ascending-dose study to assess the safety and tolerability of Pyruvate Injection in elderly male and female volunteers (GE-101-003)

The primary objective of this study was to determine the safety of Pyruvate Injection in a dose-ascending manner (0.14, 0.21, 0.28, 0.36, and 0.43 ml/kg body weight [bw]) in elderly male and female volunteers, starting with the lowest of the 5 dose levels and using appropriate placebo-treated controls.

The study was conducted at the phase 1 unit: Clinical Research Services Turku (CRST), Turku, Finland.

The safety and tolerability of 5 ascending doses of Pyruvate Injection were evaluated. Thirty subjects were enrolled into 5 dose groups. Each group contained 6 subjects (4 received Pyruvate Injection and 2 received placebo). Doses from 0.14 to 0.43 ml/kg bw of a 250 mM formulation of Pyruvate Injection were intravenously injected at a rate of 5 ml/second. At the highest dose-level, subjects received 9.4 mg/kg bw of pyruvate and 2.0 μg/kg bw of EPA. The study commenced at the lowest dose of 0.14 ml/kg bw (10 ml for a 70-kg subject).

Increasing to the next dose level occurred only after the safety data from the previous dose level had been properly evaluated by the Sponsor and the principal investigator and both agreed that it was safe to continue with the next dose. As there were no adverse safety findings at the lower doses the highest dose was 0.43 ml/kg bw (30 ml for a 70-kg subject).

The subjects randomized to receive placebo were given an IV injection of Sodium Chloride Solution for Infusion (0.9% weight/volume [w/v]) at an injection rate of 5 ml/second and at a volume that corresponded with the volume of the IMP administered in each dose group.

Safety assessments including baseline signs and symptoms, AEs, limited physical examinations, clinical laboratory tests (serum biochemistry and hematology), vital signs (heart rate, systolic and diastolic blood pressures, respiration rate, body temperature, and oxygen saturation), and 12-lead ECGs were performed at various time points pre- and post-administration. In addition, the site of injection was monitored for evidence of local and regional reactions. Changes after administration of Pyruvate Injection were compared to changes after administration of placebo. Safety data up to and including the 2-day assessment for each dose group was reviewed before proceeding to the next dose level. During the study, safety assessments were performed by an observer who was blinded to the nature (placebo or Pyruvate Injection) of the injection.

Dose Groups of Investigational Medicinal Product and Placebo

Dose Group	Pyruvate Injection (250 mM Pyruvate + 3 μM EPA)					Placebo[a] No. of Subjects
	Dose (ml/kg bw)	Dose (ml/70 kg)	Pyruvate Dose (mg/kg bw)	EPA Dose (μg/kg bw)	No. of Subject	
1	0.14	10	3.1	0.7	4	2
2	0.21	15	4.6	1.0	4	2
3	0.28	20	6.3	1.3	4	2
4	0.36	25	7.9	1.7	4	2
5	0.43	30	9.4	2.0	4	2

bw = body weight; w/v = weight in volume; EPA = electron paramagnetic agent.
[a] = equivalent volume of saline: Sodium Chloride Solution for Infusion (0.9% w/v).

Demographics

Summary Statistics for Baseline Demographic Characteristics by Treatment Group

Variable		Dose Group					
		Pyruvate Injection					
		0.14 ml/kg N=4	0.21 ml/kg	0.28 ml/kg	0.36 ml/kg N=4	0.43 ml/kg N=4	Placebo N=10
Gender	Male	2 (50%)	2 (50%)	3 (75%)	2 (50%)	3 (75%)	3 (30%)
	Female	2 (50%)	2 (50%)	1 (25%)	2 (50%)	1 (25%)	7 (70%)
Race	Caucasian – Not Hispanic or	4 (100%)	4 (100%)	4 (100%)	4 (100%)	4 (100%)	10 (100%)
Age (Years)	Mean	62.0	65.5	62.0	68.3	67.5	64.7
	SD	3.37	3.11	1.83	5.68	4.12	7.76
	Range (min-max)	60-67	61-68	60-64	62-74	64-72	60-86
Weigh t (kg)	Mean	76.5	70.5	73.3	69.8	68.5	71.8
	SD	16.94	3.70	13.07	11.79	13.13	14.58
	Range (min-max)	61-94	66-74	62-91	54-81	50-81	58-100
Height (cm)	Mean	172.3	162.0	171.5	167.8	167.3	169.5
	SD	12.97	6.68	4.43	8.54	11.59	7.56
	Range (min-max)	156-185	154-168	168-178	159-179	152-180	157-183
BMI (kg/m^2)	Mean	25.53	26.93	24.90	24.68	24.25	24.85
	SD	2.749	2.018	4.446	2.936	1.758	3.753
	Range (min-max)	22.3-29.0	24.7-29.3	22.0-31.5	21.4-28.4	21.6-25.5	19.9-31.8

N = Total number of subjects in that treatment group; BMI = Body mass index

Summary of All AEs by Treatment Group, Causality, Intensity, Seriousness, Withdrawals and Deaths

	Pyruvate Injection										Placebo N=10	
	0.14 ml/kg		0.21 ml/kg		0.28 ml/kg		0.36 ml/kg		0.43 ml/kg			
	No. of Subjects with an AE n (%)	No. of AEs	No. of Subjects with an AE n (%)	No. of AEs	No. of Subjects with an AE n (%)	No. of AEs	No. of Subjects with an AE n (%)	No. of AEs	No. of Subjects with an AE n (%)	No. of AEs	No. of Subjects with an AE n (%)	No. of AEs
Any AE[a]	1 (25%)	4	1 (25%)	1	3 (75%)	3	2 (50%)	4	3 (75%)	3	1 (10%)	2
Causality												
Related	1 (25%)	2	1 (25%)	1	0 (0%)	0	1 (25%)	1	2 (50%)	2	1 (10%)	1
Not related	1 (25%)	2	0 (0%)	0	3 (75%)	3	1 (25%)	3	1 (25%)	1	1 (10%)	1
Intensity												
Mild	1 (25%)	3	1 (25%)	1	3 (75%)	3	2 (50%)	4	3 (75%)	3	1 (10%)	2
Moderate	1 (25%)	1	0 (0%)	0	0 (0%)	0	0 (0%)	0	0 (0%)	0	0 (0%)	0
Serious AEs	0 (0%)	0	0 (0%)	0	0 (0%)	0	0 (0%)	0	0 (0%)	0	0 (0%)	0
Withdrawals due to AE	0 (0%)	0	0 (0%)	0	0 (0%)	0	0 (0%)	0	0 (0%)	0	0 (0%)	0
Deaths	0 (0%)	0	0 (0%)	0	0 (0%)	0	0 (0%)	0	0 (0%)	0	0 (0%)	0

N = Total number of subjects in that treatment group.
[a] Subjects may have experienced more than 1 AE.

Overall, 11 subjects (37%) experienced a total of 17 AEs. Of the 20 subjects given Pyruvate Injection, 10 subjects (50%) experienced 15 AEs. Of the 10 subjects given placebo, 1 subject (10%) experienced 2 AEs. No SAEs or deaths were reported and no subjects were withdrawn due to AEs.

Summary of AE Duration, Intensity, Relationship to Investigational Medicinal Product, and Outcome, by Treatment Group

Adverse Event	Subject Numbe	Serious (yes/no)	Time from Injection to Onset	Duration of AE	Intensity	Relation-ship to IMP	Outcome
0.14 ml/kg Pyruvate Injection							
Dyspepsia	001-0004	No	7 min	43 min	Moderate	Suspected	Resolved
Dysgeusia	001-0004	No	12 min	3 min	Mild	Suspected	Resolved
Diarrhea	001-0004	No	8 h 35 min	16 min	Mild	Not suspected	Resolved
Injection site edema	001-0004	No	9 h 20 min	Unknown	Mild	Not suspected	Resolved
0.21 ml/kg Pyruvate Injection							
Dysgeusia	001-0010	No	5 s	2 s	Mild	Suspected	Resolved
0.28 ml/kg Pyruvate Injection							
Medical device site reaction	001-0014	No	7 h 40 min	Unknown	Mild	Not suspected	Resolved
Dizziness	001-0016	No	1 d 9 h 50 min	15 min	Mild	Not suspected	Resolved
Headache	001-0018	No	9 h 30 min	1 h	Mild	Not suspected	Resolved
0.36 ml/kg Pyruvate Injection							
Headache	001-0019	No	57 min	30 min	Mild	Not suspected	Resolved
	001-0019	No	20 h 20 min	12 h 30 min	Mild	Not suspected	Resolved
Erectile dysfunction	001-0019	No	5 h 20 min	Unknown	Mild	Not suspected	Resolved
Feeling hot	001-0022	No	Immediate	1 min	Mild	Suspected	Resolved
0.43 ml/kg Pyruvate Injection							
Pharyngo-laryngeal discomfort	001-0026	No	Immediate	15 s	Mild	Suspected	Resolved
Diarrhea	001-0029	No	5 h 10 min	1 min	Mild	Not suspected	Resolved
Flushing	001-0030	No	10 s	5 s	Mild	Suspected	Resolved
Placebo							
Injection site coldness	001-0023	No	Immediate	1 min	Mild	Suspected	Resolved
Somnolence	001-0023	No	4 h 40 min	2 h	Mild	Not suspected	Resolved

d = day; h = hour; min = minute; s = second.

All but 1 of the AEs (16 of 17 AEs) was mild in intensity. One AE was of moderate intensity.

A total of 7 AEs (41%) were suspected to be related to IMP, 6 of 15 AEs (40%) in the Pyruvate Injection dose groups and 1 of 2 AEs (50%) in the placebo group. The proportion of subjects experiencing AEs classified as related to IMP appeared to be slightly higher in the 0.43 ml/kg dose group (50% of the subjects) than in the other groups (0.14 ml/kg: 25%, 0.21 ml/kg: 25%, 0.28

ml/kg: 0%, 0.36 ml/kg: 25% and placebo: 10%).

All AEs resolved before final follow-up. Treatment was given for 2 AEs. Subject 001-0014 was given hydrocortisone for a medical device site reaction and subject 001-0018 was given ibuprofen for a headache. An additional subject contact was arranged for subject 001-0004 (injection site edema). No safety concerns were raised over any of the AEs.

Safety conclusions

- Of the 30 subjects enrolled in the study, 11 subjects (37%) experienced a total of 17 AEs. Of these, 10 out of 20 subjects (50%) in the Pyruvate Injection dose groups experienced 15 AEs and 1 out of 10 subjects (10%) in the placebo dose group experienced 2 AEs. All but 1 of the AEs was mild in intensity (16, 94%). No deaths, SAEs or withdrawals due to AEs occurred during the study. A total of 7 AEs (41%) were suspected to be related to IMP, 6 of 15 AEs (40%) in the Pyruvate Injection dose groups (dyspepsia, dysgeusia - 2 events, feeling hot, pharyngeal discomfort, and flushing) and 1 of 2 AEs (50%) in the placebo group (injection site coldness). While it is apparent that the percentage of AEs attributed to IMP is higher in subjects given Pyruvate Injection than those given placebo, most of these events (e.g., dysgeusia, feeling hot, pharyngeal discomfort and flushing) were mild, short-lasting symptomatic reactions that are commonly reported after intravenous injection of 10 ml or more of other types of contrast agents, e.g., those used for X-ray and MRI examinations.

- There was overall stability throughout the follow-up period for all parameters, including serum biochemistry, hematology, vital signs and ECG variables. No clinically important trends or safety signals were noted.

Overall safety conclusion

In this study, the most common AEs were headache, dysgeusia and diarrhea, each experienced by 10% of the subjects who received Pyruvate Injection and none of the subjects who received placebo. In a previous study in young healthy volunteers, the most common AEs were flushing and dizziness (see study GE-101-001 above). Given that the number of subjects included in each study was low and that all these events were mild, short-lasting symptomatic events that are commonly seen in clinical studies with intravenous contrast agents, this difference is neither surprising nor concerning.

Throughout the study, serum biochemistry, hematology, vital signs and ECG variables showed overall stability and no dose-related tendencies. No clinically important trends or safety signals were noted.

While it is apparent that the percentage of AEs attributed to IMP is higher in subjects given Pyruvate Injection than those given placebo, most of these events (e.g., dysgeusia, feeling hot, pharyngeal discomfort and flushing) were mild, short-lasting symptomatic reactions that are commonly reported after intravenous injection of 10 ml or more of other types of contrast agents, e.g., those used for X-ray and MRI examinations. Therefore, this difference does not raise any concerns.

6.3.3 A Phase 1/2, Metabolic Imaging of patients with prostate cancer using hyperpolarized [1-13C]pyruvate (NCT01229618)

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The primary objective of this study of the hyperpolarized [1-13C]pyruvate performed at UCSF (Nelson, 2013), was to demonstrate the safety and feasibility of hyperpolarized [1-13C]pyruvate (HP-[1-13C]pyr) injections in men with prostate cancer. The safety of hyperpolarized [1-13C]pyr injection was assessed in a dose-ascending manner (0. 14, 0.28, 043 ml/kg body weight), six patients at each dose, see Table below. After establishing the maximum of dose level (0.43 ml/kg body weight), the second phase of the study was to refine MR methods as to evaluate the kinetics of delivery to the prostate and assess differences in [1-13C]lactate/[1-13C]pyruvate for regions of cancer versus other tissues in 13 patients.

Table 5. Summary of study design NCT 01229618.

I got Study Component	Dose (ml/kg)	N Patients	MR Acquisition
Phase 1: Dose escalation	0.14	3	1D dynamic MRSI
		3	3D MRSI
	0.28	3	1D dynamic MRSI
		3	3D MRSI
	0.43	3	1D dynamic MRSI
		3	2D MRSI
Phase 2: Refining MR methods	0.43	5	2D dynamic MRSI
		3	2D MRSI
		5	3D MRSI

The study was conducted at the University of California, San Francisco (UCSF). 31 eligible patients were recruited, with untreated, biopsy proven localized prostate cancer, no significant history of cardiac or pulmonary disease and adequate baseline organ function. Each received a multi-parametric 1H MR staging examination before the hyperpolarized MR study to confirm that they had observable lesions and ensure that they were familiar with standard imaging procedures.

For phase 1 of the study, the commonly used 3 + 3 dose escalation clinical trial design was modified to enroll six patients at each dose: three to monitor the kinetics of delivery and three to evaluate the spatial distribution of metabolism in tumor versus other tissues. Three dose levels were administered (0.14, 0.28, or 0.43 ml/kg actual body weight of 250 mM pyruvate solution). Patients underwent continuous electrocardiogram monitoring during and for 10 min after the injection, as well as at baseline, 1 hour, and 2 hours after the injection. Clinical monitoring was undertaken for 2 hours after injection, with clinical and laboratory assessments performed at 24 hours and 7 days.

The hyperpolarized [1-13C]-pyruvate was produced under aseptic conditions using a DNP polarizer located in a clean room adjacent to the 3 T MR scanner. The formulation comprised [1-13C]pyruvate (22.0 mg/ml), sodium (4.1mg/ml), tris (12.1mg/ml), EDTA (0.1 mg/ml), and tris{8-carboxyl-2,2,6,6-tetra[2-(1-methoxyethyl)]benzo(1,2-d:4,5 d)bis(1,3)dithiole-4-yl}methyl (radical) (4.6 mg/ml) in sterile water for injection. All components were manufactured according to current Good Manufacturing Practices and were provided in sterile single-use packaging by GE Healthcare. After dissolution and neutralization, column filtration removed the EPA, passed the hyperpolarized

agent through a 0.22-mm sterile filter and collected it in a drug product vessel. The automated QC system provided by GE Healthcare required 3 ml for testing. An investigational pharmacist monitored the process and released the sample for injection if it met the specifications defined in the IND: pH in the range of 6.7-8.0, temperature in the range 25-37°C, polarization not less than 15% and residual EPA concentration no higher than 3.0 µg. The drug product vessel was then transferred to the scan room and the appropriate volume drawn into a syringe for manual injection.

For the 31 samples injected into patients, the average polarization was 17.8% (range, 15.9 to 21.1), pH was 7.6 (range, 7.3 to 8.0), temperature was 32.4°C (range, 28.8 to 36.4), and volume was 51.9 ml (range, 31.9 to 53.5). QC criteria were defined as follows: polarization to be not less than 15%, pH in the range of 6.7 to 8.0, sample temperature in the range of 25° to 37°C, and residual EPA concentration no higher than 3.0 mg. The dissolution took an average of 17.8 s (range, 5 to 30), the QC process 13.1 s (range, 10 to 19), delivery through the hatch into the scan room 21.8 s (range, 11 to 30), and injection 14.9 s (range, 6 to 28). Overall, this gave an average of 67.6 s (range, 43 to 88) to deliver the agent to the subject. The mean injection volumes for the patients studied at each dose were 11.8 ml (range, 10 to 14), 26.8 ml (range, 22 to 33), and 34.5 ml (range, 29 to 46), with mean injection times of 8.5 s (range, 6 to 10), 14.8 s (range, 10 to 27), and 12.5 s (range, 1 to 28), respectively. Variation in injection times reflected differences in the volume delivered, as well as in the time for drawing the material from the drug product vessel into a syringe and performing the manual injection.

Toxicities were graded with CTCAE v4.0 criteria. A DLT was defined as an event of grade 2, 3, or 4 that was attributable to the agent and occurred within 7 days after the imaging examination. The stopping rule in each cohort of six subjects was as follows: if 0 or 1 dose DLT were observed, the study would proceed to the next dose level; if two DLTs were observed, that dose would become the maximum tolerated dose; and if more than two DLTs occur, the next lower dose would be considered as the maximum tolerated dose.

Vital signs were monitored before and immediately after the imaging examination, with subsequent telephone follow-ups over a period of 7 days to check for evidence of adverse events. In phase 1 of the study, there were a total of 10 adverse events in eighteen patients, see tTable below. These were all considered mild events and were classified as grade 1 by Common Terminology Criteria for Adverse Events (CTCAE) v4.0. criteria. The highest dose of [1-13C]pyruvate (0.43 ml/kg) was selected for further study based on the higher signal-to-noise ratio (SNR) of hyperpolarized [1-13C]pyruvate that was observed. In phase 2, there were an additional 10 events observed in five patients, but, again, none of them were considered dose-limiting toxicities (DLTs). The single episode of dizziness that was seen in one patient during phase 2 was attributed to extra dosing of atenolol, which was used by the subject to reduce anxiety rather than the hyperpolarized agent. There was one episode of grade 2 diarrhea reported in the phase 2 component, which was attributed to an enema that the patient received.

Table 6. NCT01229618. Observed adverse events and grade

Study component	Dose level (ml/kg)	*n* patients total	*n* patients with events	*n* events total	Symptom reported	*n* events	Grade
Phase 1	0.14	6	3	3	Orange urine	1	1
					Pharmaceutical smell	1	1
					Pruritus	1	1
	0.28	6	2	2	Cold sensation with injection	1	1
					Dysgeusia (distorted taste)	1	1
	0.43	6	3	5	Sore throat	1	1
					Hypocalcemia	1	1
					Hypokalemia	1	1
					Dysgeusia (distorted taste)	2	1
Phase 2	0.43	13	5	10	Dizziness*	1	1
					Dysgeusia (distorted taste)	2	1
					Fatigue	1	1
					Hypotension	1	1
					Nausea	1	1
					Pain (headache)	1	1
					Smell Change	2	1
					Diarrhea*	1	2

* On independent review attributed to causes other than the hyperpolarized injection.

Once either the maximum dose or the maximum tolerated dose cohorts were completed, phase 2 of the study design included an expansion cohort to optimize the imaging protocol and explore the biological variability in delivery, transport, and metabolism of the agent.

For ^{13}C MR examination, an intravenous catheter was placed, and the patient was positioned in a clinical 3-T MR scanner (GE Healthcare). For anatomic imaging, the 1H body coil was used for transmission with a pelvic phased array and custom-designed 1H/13C endorectal coil for reception, see Figure 4 below. For 13C data, a bore-insertable volume coil that was hinged like a clamshell to facilitate patient entry was used for transmission with the 13C channel of the endorectal coil for reception. Anatomic images comprising a scout, sagittal, and axial T2-weighted fast spin echo sequences were acquired first, followed by 13C signal calibration that used the signal from a sealed standard housed within the endorectal coil containing 600 µl of 8 M 13C-urea. Once the appropriate scan parameters had been defined, the operators in the clean room started the dissolution. If the sample satisfied the tests imposed by the QC system and the pharmacist who was monitoring the study gave their approval, the formulation was injected into the patient at ~5 ml/s and 13C data were obtained.

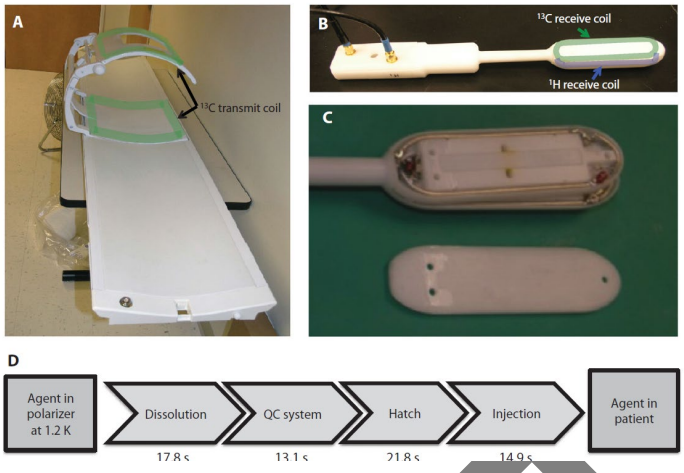

Figure 4. ^{13}C coil setup and the schematic steps for the delivery of hyperpolarized [1-13C]pyruvate.

(A to C) ^{13}C transmit coil (A) and endorectal 1H/13C receiver coil (B) used for acquiring data. The location of the coils is outlined on (A) and (B), with the layers inside the endorectal coil being seen in (C). The dimensions of the elements in the endorectal coil were 4 inches × 1 inches, with the total length of the coil being 12 inches. (D) Steps involved in transferring the hyperpolarized agent from the polarizer to the patient, and mean times required for each of them.

Spatially localized dynamic 13C spectroscopic imaging monitored delivery, transport, and metabolism of hyperpolarized [1-13C]pyruvate. Single–time point 2D or 3D acquisitions were used to obtain arrays of 13C spectra from the prostate and surrounding tissues in 8 to 12 s. The initial sequence parameters were chosen based on studies in murine and dog models but were further refined as the study progressed in terms of the start times for acquiring MR data and the flip angle schemes used.

The analysis of the 13C MR data used specialized software developed in UCSF. Arrays of spectra were obtained by apodizing the raw data with a 10-Hz Lorentzian function in the time domain and performing a Fourier transform. For data with echo planar localization, signals from the positive and negative gradient lobes were separately reconstructed for each trajectory with regridding of samples on the ramps. The spectral arrays were then zero- and first-order phase corrected. Quantification of individual spectra used automatic phasing, baseline subtraction, and frequency correction. The heights and areas of spectral peaks were estimated and used to generate metabolite images and/or curves of the time course of changes in [1-13C]lactate and [1-13C]pyruvate. The spectral arrays and metabolite images were directly correlated with anatomic images that were acquired within the same examination. For comparison purposes, regions of prostate cancer were identified as areas with concordant positive TRUS-guided biopsy and MRI abnormality within the same sextant of the prostate. Visual comparisons of the locations of regions with elevated lactate/pyruvate on the 13C images were made with the results from the MR staging examination using anatomic images from the two studies as a reference to see whether similar regions were identified as having abnormalities. For voxels where the SNR of the dynamic data was sufficient, the curves of lactate and pyruvate were fit with the two-compartment model that was developed and applied in previous preclinical studies.

The purpose of the 1D spatially localized dynamic data was to establish the time course of delivery of the agent. The acquisition provided spectra from an axial slab that covered the prostate and surrounding tissues and applied echo planar encoding from slices in the right-left direction at a 3-s

time resolution starting at the end of the injection from a 36- to 60-mm axial slice encompassing the prostate. Echo planar localization was applied in the right-left direction at 10-mm resolution and with echo time (TE)/repetition time (TR)/flip angle of 2 ms/3 s/10°. These dynamic data demonstrated reproducible delivery of hyperpolarized [1-13C]pyruvate to the prostate and its conversion to hyperpolarized [1-13C]lactate. Representative 1D dynamic 13C spectra are shown below:

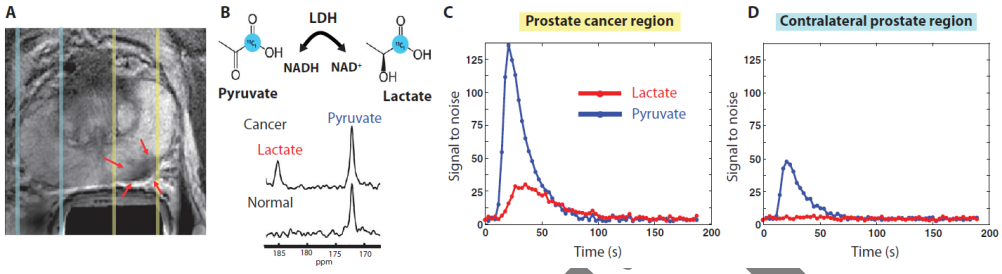

Figure 5. 1D 13C dynamic MRSI data: Images are from a representative patient with a current PSA of 12.2 ng/ml, a small volume of biopsy-proven Gleason grade 4 + 3 prostate cancer in the left midgland, and who received the lowest dose (0.14 ml/kg) of hyperpolarized [1-13C]pyruvate. (A) Axial T2-weighted image showing slices (dashed lines) obtained from 1D spectral localization. The slice that overlaps the left prostatic peripheral zone (right side of image) contained a small focus of reduced T2 signal intensity corresponding to the region of biopsy-proven cancer (red arrows). The slice overlapping the right peripheral zone (left side of image) contains only normal prostate tissue. (B) Flux of [1-13C]pyruvate to [1-13C]lactate catalyzed by LDH (top). Dynamic 13C spectra were obtained from the same patient in (A) at 36 s after injection of hyperpolarized [1-13C]pyruvate (bottom). The cancer spectrum demonstrated a lactate SNR of 25 owing to a high flux of hyperpolarized [1-13C]pyruvate to [1-13C]lactate. (C) Plot of 1D localized dynamic hyperpolarized pyruvate and lactate data from the slice that overlapped the region of prostate cancer. (D) Plot of 1D localized dynamic hyperpolarized pyruvate and lactate data from the slice that overlapped a contralateral region of the prostate.

To resolve the concern with the 1D localized dynamic MRSI data that the contribution from hyperpolarized signals in tissues outside the prostate could confound the interpretation of estimated parameters, 2D spatially localized dynamic MRSI data were acquired in five patients in a phase 2 study. The 2D localized dynamic MRSI spectra were obtained every 5 s starting at 5 s after the end of injection from a 12- to 40-mm axial slice with eight-phase encodes in one dimension and with 18-step echo planar localization in the other dimension to provide 10-mm in-plane resolution. The radio frequency pulses that were used applied either a 10° flip angle for all metabolites or a specially designed pulse that gave a flip angle of 10° for pyruvate and 20° for lactate. The TE/TR was 3/125 ms. Representative 2D dynamic 13C spectra are shown below:

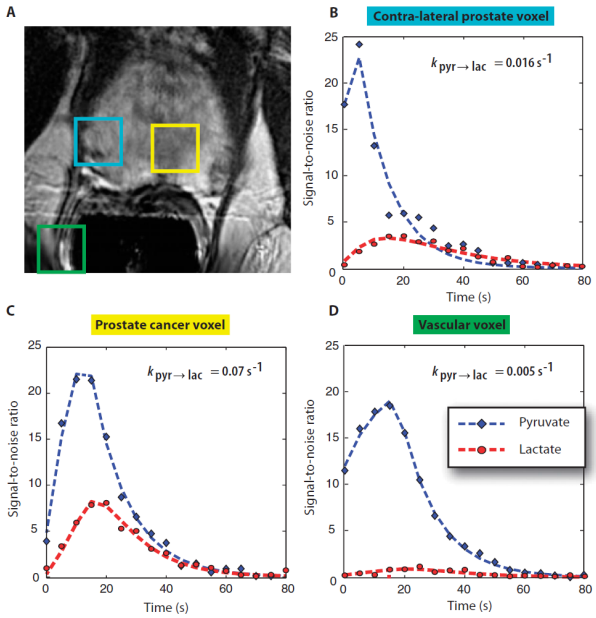

Figure 6. 2D 13C dynamic MRSI data: Images are from a representative patient with a current PSA of3.6 ng/ml, who had biopsy-proven prostate cancer in the left apex (Gleason grade 3 + 4) and received the highest dose of hyperpolarized [1-13C]pyruvate (0.43 ml/kg). (A) A focus of mild hypointensity can be seen on the T2-weighted image, which was consistent with the biopsy findings. (B to D) 2D localized dynamic hyperpolarized [1-13C]pyruvate and [1-13C]lactate from spectral data that were acquired every 5 s from voxels overlapping the contralateral region of prostate (turquoise), a region of prostate cancer (yellow), and a vessel outside the prostate (green).

To compare the relative levels of [1-13C]lactate and [1-13C]pyruvate in regions of tumor versus normal prostate and surrounding tissue, 2D single–time point spatially localized MRSI data were acquired. The 2D single–time point MRSI, spectra were obtained from a 10- to 20-mm axial slice with 12 by 12–phase encodes and 7-mm in plane resolution with a progressive flip angle and TE/TR of 3/85 ms. The acquisition time was 12 s and started 25 to 33 s after the end of the injection. The variation in slice thickness was required to cover the region of putative tumor. Representative 2D single-point 13C MRSI are shown below:

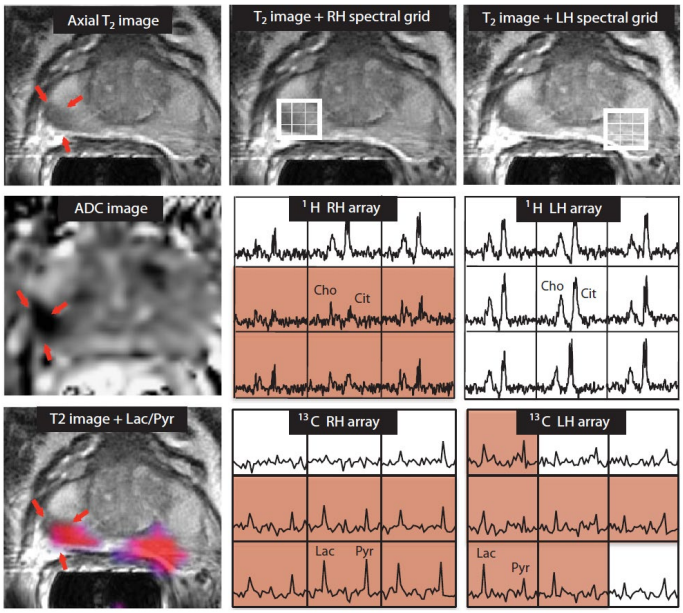

Figure 7. 2D single–time point MRSI data: Images were obtained from a patient with serum PSA of 9.5 ng/ml, who was diagnosed with bilateral biopsy-proven Gleason grade 3 + 3 prostate cancer and received the highest dose of hyperpolarized [1-13C]pyruvate (0.43 ml/kg). The axial T2-weighted image shows a unilateral region of reduced signal intensity (red arrows), which is consistent with a reduction in the corresponding ADC. The 1H spectral arrays supported these findings, with voxels with reduced citrate and elevated choline/citrate (highlighted in pink) on the right side of the gland and voxels with normal metabolite ratios on the left side. The 13C spectral arrays show voxels with elevated levels of hyperpolarized [1-13C]lactate/[1-13C]pyruvate (highlighted in pink) on both the right and left sides of the prostate. The location of colored regions in the metabolite image overlay had a ratio of [1-13C]lactate/[1-13C]pyruvate greater than or equal to 0.6.

To obtain an accurate estimate of the ratio of [1-13C]lactate/[1-13C]pyruvate for tumor versus normal tissue, 3D single–time point MRSI were acquired. A 3D array of spectra was obtained from a 43- to 120-mm axial slice with 12 by 8 to 12–phase encodes and 18 echo planar frequency encodes. The in-plane resolution was 7 mm and the through-plane resolution was 7 to 15 mm, with a progressive flip angle and TE/TR of 3/85 to 125 ms. Variations of the in-plane slice thickness, number of phase encodes, and spatial resolution were driven by differences in the size of the prostate and the spatial extent of the region of tumor. The acquisition time was 8 to 12 s, and it started 25 to 33 s after the end of the injection. Representative 3D single-point 13C MRSI are shown below:

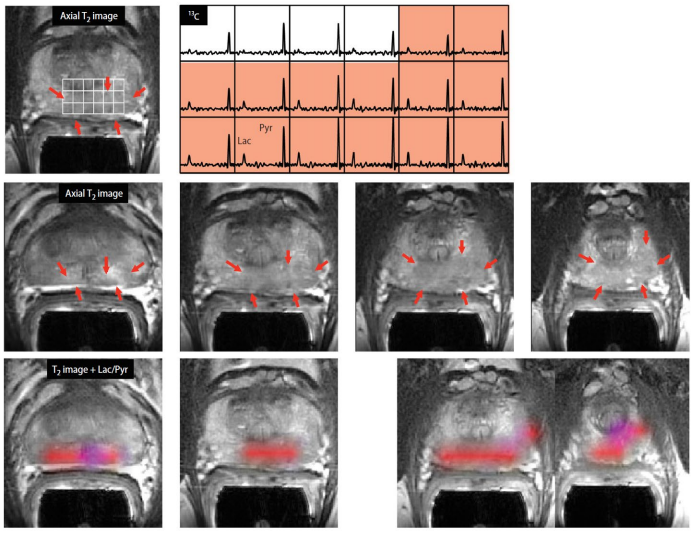

Figure 8. 3D single–time point localized MRSI data: Images were obtained from a patient who had a serum PSA of 4.5 ng/ml, was originally diagnosed with bilateral biopsy-proven Gleason grade 3 + 3 prostate cancer, and received the highest dose of hyperpolarized [1-13C]pyruvate (0.43 ml/kg). Upper panel: axial T2-weighted images and corresponding spectral array with the area of putative tumor highlighted by pink shading. A region of tumor was observed on the T2-weighted images (red arrows), as well as on ADC images and 1H MRSI data. A region of relatively high hyperpolarized [1-13C]lactate was observed in the same location as the abnormalities that had been observed on the multi-parametric 1H staging exam. Lower panels: axial T2 images with and without metabolite overlays for different axial slices from the same patient. The colored regions in these overlays have a ratio of [1-13C]lactate/[1-13C]pyruvate ≥0.2.

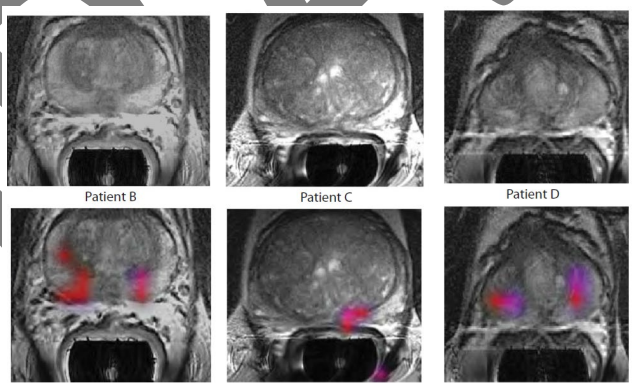

Figure 9. Patient's representative 3D single–time point MRSI data: The axial T2-weighted images and overlays of hyperpolarized [1-13C]lactate/[1-13C]pyruvate. All three of patients had biopsy-proven Gleason grade 3 + 3 prostate cancer and received the highest dose of hyperpolarized [1-13C]pyruvate (0.43 ml/kg). Patient B had a current PSA of 5.1 ng/ml, patient C had a PSA of 9.8 ng/ml, and patient D had a PSA of 1.9 ng/ml.

6.4 Literature reported studies

6.4.1 Hyperpolarized 13C Metabolic MRI of the Human Heart: Initial Experience (NCT02648009)

In this report by Cunningham (2016) four healthy subjects underwent conventional proton cardiac magnetic resonance imaging followed by ^{13}C imaging and spectroscopic acquisition immediately after intravenous administration of a 0.1 mmol/kg dose of hyperpolarized [1-^{13}C]pyruvate. All subjects tolerated the procedure well with no adverse effects reported ≤1month post procedure. The [1-^{13}C]pyruvate signal appeared within the chambers but not within the muscle. Imaging of the downstream metabolites showed ^{13}C -bicarbonate signal mainly confined to the left ventricular myocardium, whereas the [1-13C]lactate signal appeared both within the chambers and in the myocardium. The mean ^{13}C image signal:noise ratio was 115 for [1-^{13}C]pyruvate, 56 for ^{13}C -bicarbonate, and 53 for [1-^{13}C]lactate.

These results represent the first 13C images of the human heart. The appearance of ^{13}C -bicarbonate signal after administration of hyperpolarized [1-13C]pyruvate was readily detected in this healthy cohort (n=4). This shows that assessment of pyruvate metabolism in vivo in humans is feasible using current technology.

6.4.2 Hyperpolarized 1-[13C]-Pyruvate Magnetic Resonance Imaging Detects an Early Metabolic Response to Androgen Ablation Therapy in Prostate Cancer

This report by Aggarwal (2017) describes the first results demonstrating the metabolic response to androgen deprivation therapy (ADT) using HP [13C]-pyruvate MRSI. A patient with metastatic prostate cancer was imaged at base line and 6weeks after initiation of ADT. The baseline scan, with markedly elevated lactate peaks within tumor containing voxels. The post therapy scan demonstrated nearly complete abrogation of elevated HP lactate peaks on HP 13C MRI, supporting the ability of HP 13C MRI to detect early metabolic responses before such a response can be ascertained using standard radiographic criteria. Concordant with these findings, the patient subsequently achieved a marked clinical response, with an undetectable serum PSA nadir at 6 mo after ADT initiation.

6.4.3 Quantifying normal human brain metabolism using hyperpolarized [1–13C] pyruvate and magnetic resonance imaging

This report by Grist (2019) studied the cerebral metabolism of intravenously injected hyperpolarized [1–13C]pyruvate in the brain of healthy human volunteers for the first time. Dynamic acquisition of 13C images demonstrated 13C-labeling of both lactate and bicarbonate, catalyzed by cytosolic lactate dehydrogenase and mitochondrial pyruvate dehydrogenase respectively. This demonstrates that both enzymes can be probed *in vivo* in the presence of an intact blood-brain barrier: the measured apparent exchange rate constant (kPL) for exchange of the hyperpolarized 13C label between [1–13C]pyruvate and the endogenous lactate pool was 0.012 _ 0.006 s^{-1} and the apparent rate constant (kPB) for the irreversible flux of [1–13C]pyruvate to [13C]bicarbonate was 0.002 - 0.002 s^{-1}. Imaging also revealed that [1–13C]pyruvate, [1–13C] lactate and [13C]bicarbonate were significantly higher in gray matter compared to white matter. Imaging normal brain metabolism with

hyperpolarized [1–13C]pyruvate and subsequent quantification, have important implications for interpreting pathological cerebral metabolism in future studies.

6.5.4 Non-Invasive *In Vivo* Assessment of Cardiac Metabolism in the Healthy and Diabetic Human Heart Using Hyperpolarized 13C MRI

This study by Rider (2020) recorded changes in cardiac metabolism in the healthy and diseased human heart in participants with and without type 2 diabetes (T2DM).
Methods and Results: Thirteen people with type 2 diabetes (HbA1c 6.9±1.0%) and 12 age-matched healthy controls underwent assessment of cardiac systolic and diastolic function, myocardial energetics (31P-MRS) and lipid content (1H-MRS) in the fasted state. In a subset (5 T2DM, 5 control), hyperpolarized [1-13C]pyruvate MR spectra were also acquired and in five of these participants (3 T2DM, 2 controls), this was successfully repeated 45 minutes after a 75g oral glucose challenge. Downstream metabolism of [1-13C]pyruvate via pyruvate dehydrogenase (PDH, [13C]bicarbonate), lactate dehydrogenase ([1-13C]lactate) and alanine transaminase ([1-13C]alanine) was assessed. Metabolic flux through cardiac PDH was significantly reduced in the people with type 2 diabetes (Fasted:0.0084±0.0067[Control] vs. 0.0016±0.0014[T2DM], Fed:0.0184±0.0109 vs. 0.0053±0.0041, p=.013). In addition, a significant increase in metabolic flux through PDH was observed after the oral glucose challenge (p<.001). As is characteristic of diabetes, impaired myocardial energetics, myocardial lipid content and diastolic function were also demonstrated in the wider study cohort.

This work represents the first demonstration of the ability of hyperpolarized 13C MRS to noninvasively detect and quantify metabolic alterations in cardiac metabolism in the human heart in the setting of cardiovascular disease.

6.4.5 First-in-human *in vivo* non-invasive assessment of intertumoral metabolic heterogeneity in renal cell carcinoma

This report by Tran (2019) reports the first-in-human *in vivo* non-invasive metabolic interrogation of renal cell carcinoma using hyperpolarized carbon-13 (13C) MRI in a single patient and describes the validation of *in vivo* lactate metabolic heterogeneity against multi regional *ex vivo* mass spectrometry. hyperpolarized carbon-13 (13C)-MRI provides an *in vivo* assessment of metabolism and provides a novel opportunity to safely and non-invasively assess cancer heterogeneity.

6.4.6 Hyperpolarized 13C MRI: a new horizon for noninvasive diagnosis of aggressive breast cancer.

This report by Abeyakoon (2019) describes a single case of breast cancer imaged using HP-MRI alongside correlative conventional imaging, including breast MRI. This young female with extensive Grade two ductal carcinoma. Her diagnostic work up included all the standard investigations and highlighted the importance of a multimodality approach to accurately map the anatomical extent of disease. The addition of HP-MRI to this case provided proof-of-concept for non-invasive *in-vivo* metabolic assessment of human breast tumor and the potential of identifying aggressive cancers.

6.4.7 Hyperpolarized MRI of Human Prostate Cancer Reveals Increased Lactate with Tumor Grade Driven by Monocarboxylate Transporter 1

This report by Granlund (2019) studied a cohort of 12 biopsy proven prostate cancer patients with C-13 pyruvate hyperpolarized MRI, assessing their pyruvate metabolism and the reproducibility of delivery. The time to max of pyruvate does not vary significantly within patients undergoing two separate injections or across patients. They show that lactate increases with Gleason grade. RNA sequencing data demonstrate a significant increase in the predominant pyruvate uptake transporter, monocarboxylate transporter 1. Increased protein expression was also observed in regions of high lactate signal, implicating it as the driver of lactate signal *in vivo*. Targeted DNA sequencing for actionable mutations revealed the highest lactate occurred in patients with PTEN loss. Therefore, this work identifies a potential link between actionable genomic alterations and metabolic information derived from hyperpolarized pyruvate MRI.

6.5 References:

Abeyakoon O, Latifoltojar A, Gong F, Papoutsaki M-V, Chowdhury R, Glaser M, et al. Hyperpolarised 13C MRI: a new horizon for noninvasive diagnosis of aggressive breast cancer. *BJR Case Rep* 2019; **5**: 20190026

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7. SUMMARY DATA AND GUIDANCE FOR THE INVESTIGATOR

7.1 Nonclinical Safety Information

A nonclinical program has been performed in which the safety of both pyruvate and AH111501 (Electron Paramagnetic Agent; EPA), the 2 novel drug product components of Pyruvate Injection, has been demonstrated. The program included expanded acute-dose studies in Sprague-Dawley rats, from which the No-Observed-Adverse-Effect-Levels (NOAEL) were estimated as 892 mg/kg bw for pyruvate and 17 mg/kg bw for AH111501. In an expanded acute-dose study in beagle dogs, the NOAEL for pyruvate was 446 mg/kg bw and 8.5 mg/kg bw for AH111501. The cardiovascular effects of formulations of pyruvate were studied in both conscious and pentobarbital/fentanyl-anaesthetized dogs. The conscious dog was considered the most relevant model for extrapolating results to healthy human volunteers and the anaesthetized dog as a sensitive model for extrapolating results to subjects with a compromised baroreflex function (as may be the case in elderly subjects with prostate cancer, which is one of the target populations in which use of Pyruvate Injection is planned). In addition, effects of pyruvate and AH111501 have been studied in local tolerance studies, genotoxicity studies and central nervous system studies. Based on the cardiovascular effects (vasodilatation leading to small, short-lasting but statistically significant reductions in blood pressure and compensatory increases in heart rate) observed in the most sensitive model, the NOAEL for Pyruvate Injection is 1.4 ml/kg bw (or 100 ml for a 70-kg subject) of a 250 mM formulation (equal to 31 mg/kg bw of pyruvate). AH111501 was qualified in a separate toxicology program and the lowest NOAEL was 8.5 mg/kg bw (or 595 mg for a 70-kg subject).

The available nonclinical data indicated sufficiently good safety margins to support testing the safety and tolerability of a 250 mM formulation of Pyruvate Injection containing 3 µM AH111501 (EPA) (i.e., 22.0 mg/ml of pyruvate and 4.6 µg/ml of EPA) in healthy human male and female volunteers in a phase 1 dose-escalating study up to doses of 0.57 ml/kg bw (i.e., up to 12.6 mg/kg bw of pyruvate and 2.6 µg/kg bw of EPA). The data from the nonclinical studies in conscious and anaesthetized dogs indicated that special attention should be paid to the possibility of mild cardiovascular effects occurring, like those reported when Pyruvate Injection was administered rapidly at large volumes to conscious dogs (increases in heart rate) and anaesthetized dogs (vasodilatation leading to small, short-lasting but statistically significant reductions in blood pressure and compensatory increases in heart rate).

7.2 Clinical Information

Pyruvate is reported to have several beneficial effects in humans when administered at supraphysiological concentrations (reviewed and discussed in Section 6). It appears to hold promise as a safe, effective ionotropic agent for treatment of hearts that have been reversibly injured (stunned) by ischemia/reperfusion stress. Pyruvate enhancement of cardiac function may result from one or more of the following mechanisms: increased cytosolic ATP phosphorylation potential and Gibbs free energy of ATP hydrolysis, enhanced sarcoplasmic reticular calcium ion uptake and release, decreased cytosolic inorganic phosphate concentration, oxyradical scavenging via direct neutralization of peroxides and/or enhancement of the intracellular glutathione/NADPH antioxidant system, and/or closure of mitochondrial permeability transition pores (for review, see Mallet [2002]). Pyruvate infusion in patients with alcoholic liver disease has also showed good therapeutic effectiveness, possibly due to the rapid gain of ATP and GTP, required to redress defective cells, and to the antioxidant action of pyruvate [Petkova et al., 2000]. However, in all the studies reported, various formulations of pyruvate have been injected at substantially lower rates

and generally much higher overall doses than will be applied in the current and subsequent studies with Pyruvate Injection.

7.3 Possible Adverse Effects

As with all clinical studies with unregistered investigational medicinal products that have been tested in a limited number of human volunteers previously, study personnel must continue to remain vigilant for the occurrence of adverse events (AEs), particularly those that may be life-threatening. Personnel who are trained in the acute management of anaphylaxis and other emergencies and who have access to appropriate clinical supplies and equipment must be immediately available while the subject is confined to the clinical study unit. Treatment of serious AEs should be primarily supportive of vital functions.

The data from the nonclinical studies in conscious and anaesthetized dogs indicated that special attention should be paid to the possibility of mild cardiovascular effects occurring, like those reported when Pyruvate Injection was administered rapidly at large volumes to conscious dogs (increases in heart rate) and anaesthetized dogs (vasodilatation leading to small, short-lasting but significant reductions in blood pressure and compensatory increases in heart rate).

In the 2 placebo-controlled, ascending-dose clinical studies in humans that have been performed in young and elderly (≥60 years old) male and female volunteers (GE-101-001 and GE-101-003), a total of 66 subjects were evaluated for safety – 44 received Pyruvate Injection and 22 received placebo. In study GE-101-001, doses up to 0.57 ml/kg of Pyruvate Injection were assessed. The study was temporarily stopped by the sponsor after completion of dosing for the 0.57 ml/kg dose group due to the occurrence of non-serious AEs in 2 subjects ('unresponsiveness' in subject 001-0026 and 'flushing' accompanied by changes in blood pressure and heart rate in subject 001-0030) that the principal investigator considered to be concerning and related to the administration of Pyruvate Injection. These events occurred in 2 of the 4 subjects in that dose group who received Pyruvate Injection. The sponsor decided not to proceed with the planned dose escalation to 0.71 ml/kg. The protocol was amended to repeat the 0.43 ml/kg dose group in place of the planned 0.71 ml/kg dose group to confirm that 0.43 ml/kg was as well tolerated in a second placebo-controlled cohort of 6 healthy volunteers as it was in the first one. Additional safety monitoring was considered but was deemed unnecessary. In study GE-101-003, doses up to 0.43 ml/kg of Pyruvate Injection were assessed.

Overall, 29 of the 66 subjects (44%) experienced a total of 58 AEs. Of the 44 subjects given Pyruvate Injection, 25 subjects (57%) experienced 50 AEs. Of the 22 subjects given placebo, 4 subjects (18%) experienced 8 AEs. No SAEs or deaths were reported, and no subjects were withdrawn due to AEs. All 8 AEs in the placebo subjects were mild in intensity. Forty-five (90%) of the 50 AEs in the Pyruvate Injection subjects were mild in intensity and 4 (8%) were moderate in intensity. The AE of severe intensity (flushing) and 3 of those of moderate intensity (2 cases of increased heart rate and 1 of unresponsiveness to stimuli) occurred in the0.57 ml/kg dose group and were suspected to be related to Pyruvate Injection. The other 'moderate' event was dyspepsia in a subject that received the 0.14 ml/kg dose of Pyruvate Injection. A total of 37 AEs (64%) were suspected to be related to IMP, 35 of 50 AEs (70%) in the Pyruvate Injection dose groups and 2 of 8 AEs (25%) in the placebo group. Since neither the subjects nor the safety observers were aware of which IMP had been given, this indicates that many of the events suspected of being related to Pyruvate Injection were truly related to the administration of this IMP. The most frequently reported individual AEs were flushing (6 subjects, 7 AEs),

dizziness (6 subjects, 6 AEs), dysgeusia (4 subjects, 5 events) and feeling hot (5 subjects, 5 AEs). Flushing and feeling hot were reported only in subjects receiving Pyruvate injection, and dizziness and dysgeusia were mainly reported in subjects receiving Pyruvate Injection (9 out of 11 AEs), indicating that these events are in most cases linked to the administration of Pyruvate Injection. Throughout both studies, serum biochemistry, hematology, urinalysis, vital signs and ECG variables showed overall stability and no dose-related tendencies. No clinically important trends or safety signals were noted.

A phase 1 study on hyperpolarized [1-13C]pyruvate injection was completed in 31 subjects with prostate cancer (NCT01229618). This was an ascending-dose study to access the safety and tolerability and imaging potential of hyperpolarized [1-13C] pyruvate Injection via 13C imaging (13C MRI) and 13C MR spectroscopic imaging (13C MRSI). Three dose levels of hyperpolarized [1-13C]pyruvate (0.14 ml/kg, 0.28 ml/kg and 0.43 ml/kg) with a minimum of 6 patients at each dose level were administered via single IV injection followed by MR imaging scans. The first 3 subjects underwent dynamic 13C MRI to define the kinetics of delivery and metabolism of hyperpolarized [1-13C]pyruvate. The second 3 subjects underwent 13C MRSI to obtain 3-dimensional (3-D) spatial information about metabolism of hyperpolarized [1-13C]pyruvate in regions of the prostate with and without cancer involvement. In phase 1 of the study, there were a total of 10 adverse events in eighteen patients. These were all considered mild events and were classified as grade 1 by Common Terminology Criteria for Adverse Events (CTCAE) v4.0.criteria. The highest dose of [1-13C]pyruvate (0.43 ml/kg) was selected for further study based on the higher signal-to-noise ratio (SNR) of hyperpolarized [1-13C]pyruvate that was observed. In phase 2, there were an additional 10 events observed in five patients, but, again, none of them were considered dose-limiting toxicities (DLTs). The single episode of dizziness that was seen in one patient during phase 2 was attributed to extra dosing of atenolol, which was used by the subject to reduce anxiety rather than the hyperpolarized agent. There was one episode of grade 2 diarrhea reported in the phase 2 component, which was attributed to an enema that the patient received.

7.4 Reference Safety Information for the assessment of expectedness of serious adverse reactions

At this time, there are no expected serious adverse events (SARs). No serious adverse events have been reported with C-13 hyperpolarized pyruvate injection.

7.5 Conclusion

In conclusion, doses of Pyruvate Injection up to 0.43 ml/kg were equally well tolerated by young and elderly healthy male and female volunteers and there was no evidence of the types of changes seen in preclinical studies soon after injection in conscious and anaesthetized dogs (described above in section 7.3). Based on these findings, it is justified to assess the safety and imaging characteristics of hyperpolarized Pyruvate Injection in future clinical studies in patients with proven prostate cancer.

While it is apparent Pyruvate Injection-related AEs such as flushing, feeling hot, dysgeusia, and dizziness can be expected to occur in future studies, most of these events will present as mild in intensity, short-lasting symptomatic reactions. Moreover, these types of events are commonly reported after intravenous injection of approximately 10 ml or more of other types of contrast agents, e.g., those used for X-ray and MRI examinations.