Using Quantitative γH2AX and H2AX ELISA for Monitoring DNA Damage Induced by Chemotherapeutic Agents and Irradiation Exposure

Date/Time	5/30/2015 8:00AM - 11:30AM
Location	S Hall A; Poster Board #275
Session	Developmental Therapeutics—Clinical Pharmacology and Experimental Therapeutics

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Abstract

Background: Gamma-H2AX (γH2AX) is a biomarker for DNA double-strand breaks and programmed cell death, but variable relative amounts of H2AX in different samples causes ambiguity in the meaning of the γH2AX level unless it is related to total H2AX levels. We developed a 96-well plate-based ELISA for quantifying γH2AX and H2AX levels in crude extracts of tumor cells, CTCs and biopsy tissues and are validating it for applications in irradiation exposure monitoring and in pharmacodynamic evaluation of anti-cancer agents.

Methods: The ELISA was used to analyze extracts of several NCI60 tumor cell lines that had been exposed to a variety of agents, including ionizing radiation, inhibitors of Top1 (CPT, SN-38, Topotecan), PARP (ABT-888, AZD-2281, MK-4827) and ATR (VE-821, VE-822, AZD-6738, Compound 45, NU-6027), and their combinations. Combination regimens of CPT-11 with PARP inhibitors (ABT-888, AZD-2281, MK-4827) were further evaluated in vivo in the A375 xenograft mouse model. Patient samples obtained for research purposes were also examined by ELISA for feasibility and utility.

Results: In vitro, dose-dependent increases in the ratio of γH2AX to H2AX were detected after escalating ionizing radiation exposure and concentration-dependent increases after Top-1 inhibitor exposure. Treating with inhibitors of PARP or ATR alone did not significantly induce γH2AX. Combinations of Top1 inhibitors with PARP or ATR inhibitors led to synergistic induction of DNA damage. Among five ATR inhibitors evaluated in combination with Top1 inhibitors, VE-822 and AZD-6738 were observed to have the highest synergy for γH2AX induction, while NU-6027 showed none. Combinations of CPT-11 with ABT-888, AZD-2281 or MK-4827 showed synergistic induction of γH2AX in A375 xenografts in vivo. Additional testing of human specimens including PBMCs, bone marrow and tumor biopsies proved the assay's clinical suitability and potential advantages.

Conclusions: A newly developed quantitative ELISA for measuring both γH2AX and H2AX is ready for clinical validation for monitoring DNA damage induced by chemotherapeutic agents or irradiation exposure.

Objectives

- To develop quantitative ELISA based immunoassay of γH2AX and H2AX for drug discovery, animal modeling and clinical monitoring of pharmacodynamics.
- PD assay applications *in vitro* screening to identify a lead combinative inhibitors of ATR and Top1.
- PD assay applications *in vivo* testing xenograft tumor biopsies after treatment of PARP and Top1 inhibitors.
- PD testing of achieved clinical specimens to show clinical applicability.

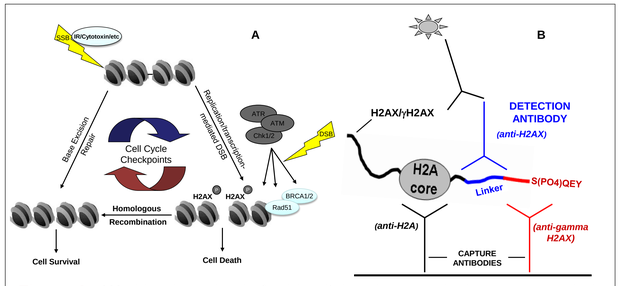

Figure 1. Working hypothesis for identifying therapeutical agents and their combinations for cell killing (A); and PD laboratory approach for monitoring pharmacodynamics using quantitative chemiluminescent enzyme-linked immunosorbant assay of γH2AX/H2AX simultaneously (B).

Methods

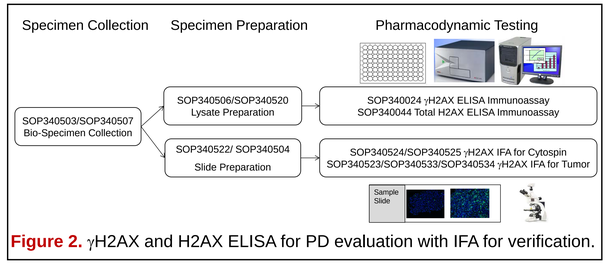

Figure 2. γH2AX and H2AX ELISA for PD evaluation with IFA for verification.

Results

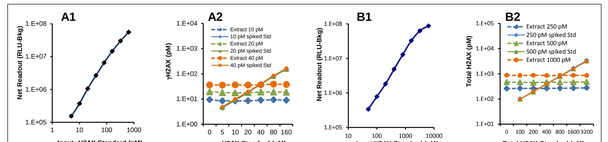

Figure 3: γH2AX standard curve for detecting various concentrations of γH2AX (pM) using ELISA. γH2AX standard (A1) and assay performance (A2); and H2AX standard (B1) and H2AX assay performance (B2).

Figure 4. Lab testing and analytic characterization. A) Quantitation of selected tumor cell lines with γH2AX/H2AX ratio (A1); γH2AX (A2) and H2AX (A3); B) IR treated cells; C) Drug treated cells; D) Spiked IR treated cells; and E) Spiked drug treated cells.

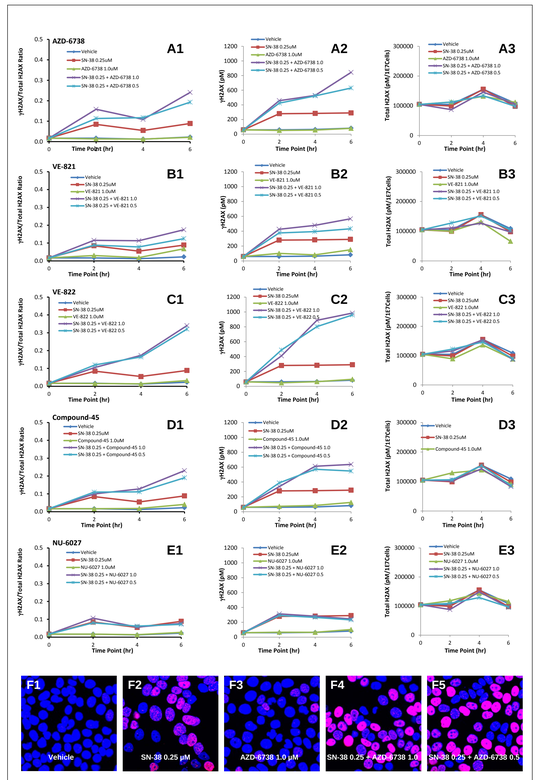

Figure 5. PD testing in vitro. MCF-7 was treated with SN38, ATRi and combinations; and quantified for ratio (1), γH2AX (2) and total H2AX (3). ATR inhibitor included AZD-6738 (A); VE-821 (B); VE-822 (C); Compound-45 (D) and NU-6027 (E). γH2AX staining of cytospin slides were used for signal confirmation as showed in AZD-6738 as examples (F).

Summary

A newly developed quantitative ELISA for measuring both γH2AX and H2AX is ready for clinical validation for monitoring DNA damage induced by chemotherapeutic agents or irradiation exposure.

References

- Kinders RJ et al (2014). *Clin Cancer Res* 20: 2578-2586.
- DCTD-NCI Biomarker Website for SOPs of PAR-IA, γH2AX-IFA and others: http://dctd.cancer.gov/ResearchResources/ResearchResources-biomarkers.htm

Acknowledgements

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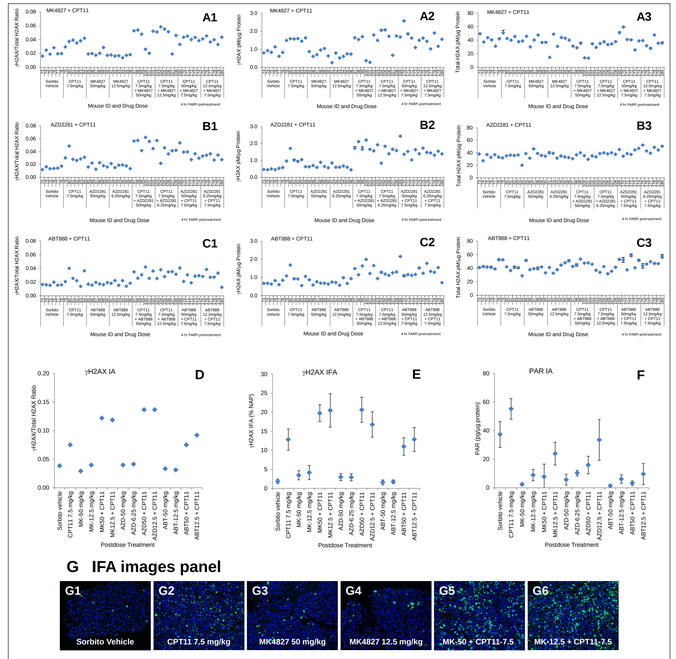

Figure 6. PD data from *in vivo* A375 xenograft models of PARPi comparison studies. Quantitative ratio (1), γH2AX (2) and total H2AX (3) of individual tumor at post-dose 6hr treatment; Average γH2AX/H2AX ratio from treatment group (D) was comparable to IFA data of γH2AX (E) with images (G). PARP inhibition was verified with PAR ELISA (F).

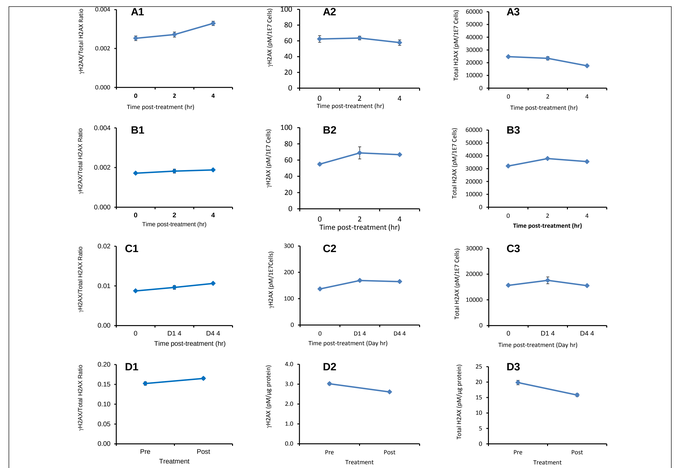

Figure 7. Clinical specimen testing of clinical specimens of PBMCs (A and B); Bone marrows (C), and tumors (D).