Introduction
Regulation of apoptotic cell death is highly important targets for cancer therapy, and developers of monoclonal antibodies (MoAbs) form a class of potential agents in clinical development. This effort was supported by the US Department of Defense (DOD) through the breast cancer research program (B153694), a collaboration between the National Cancer Institute (NCI) Breast Cancer Research Program and the University of Michigan. The purpose of this study was to evaluate the clinical potential of the multiplex platform using tumor cell lysates and included MCL1, total BAX, BAK, BCL2, BCLXL, Survivin, BCL2L1, SMAC, Oligomeric BAX, BAK, and cleaved caspase-3. The multiplex panel will facilitate quantitative measurement of these targets in terms of reproducibility, accuracy, and sample stability for clinical use.

Materials and Methods

Results

Abstract

Figure 1. Intestine: Apoptosis

Figure 2. Scheme of a protocol for clinical studies of protein levels in tumor cell lysates treated with SMAC mimetic TL32711.

Materials and Methods

Table 2. (A-C). Crossreactivity of immunoassays in Multiplex Panel

Table 3. Precision and Accuracy of Multiplex Assays

Table 4. Analytical Sensitivity of Multiplex Panel

Table 5. Linearity of Multiplex Assays

Table 6. Spike and Recovery of Multiplex Panel

Table 7. Crossreactivity of Pan Cytokine Panel

Table 8. Crossreactivity of Panel 1

Supplementary Information

Figure 3. Changes in cleaved caspase-3 (A) and Lamin B (B) in the cytoplasmic fraction of tumor xenografts treated with SMAC mimetics TL32711. Lamin B expression was monitored as a positive control of tumor growth. Lamin B levels were measured at baseline and in tumors treated for 24 hours post-treatment. Similar changes were observed in BMSCs treated with the same dosing regimen of TL32711 at 24 hours post-treatment. (p<0.05 vs. saline).

Figure 4. Changes in cleaved caspase-3 (A) and Lamin B (B) in the cytoplasmic fraction of tumor xenografts treated with SMAC mimetics TL32711. Lamin B expression was monitored as a positive control of tumor growth. Lamin B levels were measured at baseline and in tumors treated for 24 hours post-treatment. Similar changes were observed in BMSCS treated with the same dosing regimen of TL32711 at 24 hours post-treatment. (p<0.05 vs. saline).

Figure 5. Changes in cleaved caspase-3 (A) and Lamin B (B) in the cytoplasmic fraction of tumor xenografts treated with SMAC mimetics TL32711. Lamin B expression was monitored as a positive control of tumor growth. Lamin B levels were measured at baseline and in tumors treated for 24 hours post-treatment. Similar changes were observed in BMSCS treated with the same dosing regimen of TL32711 at 24 hours post-treatment. (p<0.05 vs. saline).

Figure 6. Changes in cleaved caspase-3 (A) and Lamin B (B) in the cytoplasmic fraction of tumor xenografts treated with SMAC mimetics TL32711. Lamin B expression was monitored as a positive control of tumor growth. Lamin B levels were measured at baseline and in tumors treated for 24 hours post-treatment. Similar changes were observed in BMSCS treated with the same dosing regimen of TL32711 at 24 hours post-treatment. (p<0.05 vs. saline).

Figure 7. Changes in cleaved caspase-3 (A) and Lamin B (B) in the cytoplasmic fraction of tumor xenografts treated with SMAC mimetics TL32711. Lamin B expression was monitored as a positive control of tumor growth. Lamin B levels were measured at baseline and in tumors treated for 24 hours post-treatment. Similar changes were observed in BMSCS treated with the same dosing regimen of TL32711 at 24 hours post-treatment. (p<0.05 vs. saline).

Figure 8. Changes in cleaved caspase-3 (A) and Lamin B (B) in the cytoplasmic fraction of tumor xenografts treated with SMAC mimetics TL32711. Lamin B expression was monitored as a positive control of tumor growth. Lamin B levels were measured at baseline and in tumors treated for 24 hours post-treatment. Similar changes were observed in BMSCS treated with the same dosing regimen of TL32711 at 24 hours post-treatment. (p<0.05 vs. saline).

Figure 9. Changes in cleaved caspase-3 (A) and Lamin B (B) in the cytoplasmic fraction of tumor xenografts treated with SMAC mimetics TL32711. Lamin B expression was monitored as a positive control of tumor growth. Lamin B levels were measured at baseline and in tumors treated for 24 hours post-treatment. Similar changes were observed in BMSCS treated with the same dosing regimen of TL32711 at 24 hours post-treatment. (p<0.05 vs. saline).

Figure 10. Changes in cleaved caspase-3 (A) and Lamin B (B) in the cytoplasmic fraction of tumor xenografts treated with SMAC mimetics TL32711. Lamin B expression was monitored as a positive control of tumor growth. Lamin B levels were measured at baseline and in tumors treated for 24 hours post-treatment. Similar changes were observed in BMSCS treated with the same dosing regimen of TL32711 at 24 hours post-treatment. (p<0.05 vs. saline).

Figure 11. Changes in cleaved caspase-3 (A) and Lamin B (B) in the cytoplasmic fraction of tumor xenografts treated with SMAC mimetics TL32711. Lamin B expression was monitored as a positive control of tumor growth. Lamin B levels were measured at baseline and in tumors treated for 24 hours post-treatment. Similar changes were observed in BMSCS treated with the same dosing regimen of TL32711 at 24 hours post-treatment. (p<0.05 vs. saline).

Figure 12. Changes in cleaved caspase-3 (A) and Lamin B (B) in the cytoplasmic fraction of tumor xenografts treated with SMAC mimetics TL32711. Lamin B expression was monitored as a positive control of tumor growth. Lamin B levels were measured at baseline and in tumors treated for 24 hours post-treatment. Similar changes were observed in BMSCS treated with the same dosing regimen of TL32711 at 24 hours post-treatment. (p<0.05 vs. saline).

Figure 13. Changes in cleaved caspase-3 (A) and Lamin B (B) in the cytoplasmic fraction of tumor xenografts treated with SMAC mimetics TL32711. Lamin B expression was monitored as a positive control of tumor growth. Lamin B levels were measured at baseline and in tumors treated for 24 hours post-treatment. Similar changes were observed in BMSCS treated with the same dosing regimen of TL32711 at 24 hours post-treatment. (p<0.05 vs. saline).

Figure 14. Changes in cleaved caspase-3 (A) and Lamin B (B) in the cytoplasmic fraction of tumor xenografts treated with SMAC mimetics TL32711. Lamin B expression was monitored as a positive control of tumor growth. Lamin B levels were measured at baseline and in tumors treated for 24 hours post-treatment. Similar changes were observed in BMSCS treated with the same dosing regimen of TL32711 at 24 hours post-treatment. (p<0.05 vs. saline).