Anti-proliferative effect and tumor targeting properties of SurVaxM-derived monoclonal antibodies

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ABSTRACT

Background: Survivin is an inhibitor of apoptosis protein (IAP) which is upregulated in many cancers. SurVaxM (active anti-survivin immunotherapy) is currently being evaluated in a multi-center phase II clinical trial. Circulating anti-survivin antibodies were recently detected in recurrent glioblastoma patients who received SurVaxM immunizations in a previous phase I study. Though primarily functioning as an intracellular molecule, survivin has also been identified in patient serum, and, recently, in tumor-derived exosomes.

Methods: Patients in the completed SurVaxM phase I clinical trial produced both antibody and CD8+ T cell responses against survivin. In order to gauge whether antibodies to the vaccine provided any therapeutic benefit, or might serve as potential biomarkers for SurVaxM responsiveness, we tested these antibodies in pre-clinical tumor models. Murine hybridomas derived from the SurVaxM peptide were established and purified yielding several high-affinity IgG antibodies. These antibodies were characterized for target recognition and therapeutic potential.

Results: Two of the clones with the highest affinities, 2C2 (IgG2a) and H30 (IgG1), recognized both SurVaxM peptide and the wild type survivin counterpart (ELISA assay), as well as endogenous, full-length survivin (western blotting). Survivin was detected on cells with these antibodies using both immunofluorescence and flow cytometry. Following s.c. implantation of glioblastoma (GL261) and melanoma (B16) tumors in immuno-competent C57BL/6 mice, administration of 2C2 and H30 antibodies (i.p. injection) reduced tumor growth in both models compared to an irrelevant IgG. An orthotopic intracranial glioma study showed similar anti-tumor effects when treated with SurVaxM-derived antibodies. Tumor growth was also inhibited in immuno-compromised (nude) animals treated with 2C2 or H30 antibodies, although to a lesser extent.

Conclusions: Antibodies generated in response to the SurVaxM vaccination are highly cross-reactive to survivin and provide therapeutic benefit in immuno-competent mouse tumor models. These antibodies retain some efficacy in immuno-compromised models, indicative of a direct T-cell independent effect. An anti-tumor response through anti-survivin targeted antibodies is unexpected as surface-accessible survivin expression has not yet been well described in the literature. Data presented here highlight possible avenues for investigation of mechanism(s) of action.

BACKGROUND

- SurVaxM is a synthetic long peptide, reverse neo-immunogen, that initiates an immune response to “survivin,” an inhibitor of apoptosis protein (IAP) which is expressed in glioma and other cancers.
- SurVaxM is a 15 AA long peptide-keyhole limpet hemacyanin (KLH) conjugate which triggers mid-affinity TCR’s through confirmed multi-CD8+ T cell epitopes, CD4+ T cell epitopes and B cell recognition (Antibody). SurVaxM is emulsified in Montanide ISA 51VG with GM-CSF and injected subcutaneously.
- The Phase I clinical study of SurVaxM in recurrent glioma patients showed safety and efficacy signals. 7/8 patients lived >12 months and long term survivors are alive >3 & >4 years post-vaccine.
- A Phase II multi-center trial of SurVaxM for newly diagnosed glioblastoma is underway. This study has reached 50% enrollment and an interim analysis has been performed (Q1/2017). Based upon this analysis this study will continue to completion as planned.
- Immunomonitoring of SurVaxM clinical studies found that patients were unexpectedly producing antibodies to SurVaxM. We have designed and cloned murine hybridomas producing anti-SurVaxM mAb to ascertain its potential anti-tumor efficacy in pre-clinical studies presented here.
- Although predominantly an intracellular molecule, survivin has also been identified on exosomes produced by cervical and prostatic carcinoma cells [S. Kahn et al.; PLOS ONE 7(10): e46737, 2012]. The contribution of exosomal survivin to specific effects of anti-survivin immunotherapy is actively under investigation.
- Survivin is accessible on the surface of glioma cells. It is still unclear if surface-survivin represents a particular isoform, a survivin-associated protein complex or tumor exosomes. The anti-SurVaxM antibody response should target each of these modalities.

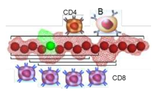
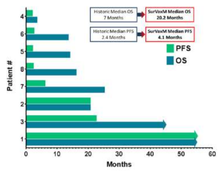


Figure 1. (above) SurVaxM structure with multiple CD8+ and CD4+ T cell epitopes highlighted. **(below)** Phase I clinical trial results for SurVaxM in recurrent glioma patients. Progression Free Survival (PFS) and Overall Survival (OS) are shown.



METHODS

Antibody generation
C57Bl/6 mice were used to produce anti-serum reactive against SurVaxM. Serum samples were obtained after 4 rounds of immunization. Several hybridoma cell lines were produced with fusion to SP2/0 myeloma cells. Purified monoclonal antibodies (mAb) of 2 subclones (2C2 & H30) are characterized here.

Elisa
Patient serum was collected and stored at -80°C. Serial dilutions of clarified serum were applied to unconjugated SurVaxM peptide, (1µg/well) on pre-coated ELISA plates in triplicate. Detection was via HRP conjugated anti-human IgG and TMB colorimetric solution.

Immunoblotting
For slot blots, purified peptides/protein (1µg/slot) were bound to nitrocellulose membrane. For Western Blotting, cell lysates were subjected to SDS-PAGE then transferred to PVDF membrane. Membranes were blocked in 5% milk in TBST and probed with antibodies at 0.2 – 0.4 µg/ml.

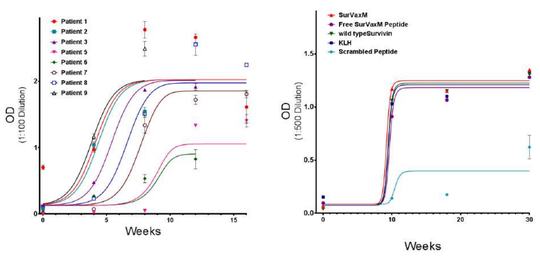
Immunofluorescence
Cells were adhered to glass coverslips, which were then washed in PBS and fixed in either 60% acetone/3.2% formaldehyde, or 4% paraformaldehyde. Paraformaldehyde-fixed slides were either washed or permeabilized with 0.1% Triton-X. Slides were subjected to blocking with 5% FBS in PBS prior to application of 2C2 and H30 at a concentration of 2-4 µg/ml, then secondary FITC-conjugated anti-mouse. Slides were viewed under a fluorescent microscope and images captured at 40X magnification.

Flow cytometry
Cells were trypsinized, recovered and incubated with antibody in either RPMI + 4% FBS or a commercial buffer, washed, and labeled with FITC-conjugated secondary ab prior to analysis. For blocking experiments, primary antibody was pre-incubated with an excess of peptide prior to cell staining.

ADCC assays
Experiments were performed using Promega mFcγRIV kit as directed by manufacturer.

RESULTS

Figure 2. Patients undergoing treatment with SurVaxM produce anti-survivin antibodies. (A) Survivin specific IgG response in SurVaxM Phase I study patients. Serum ELISA data of 1:100 dilution of patient serum is shown for 8 patients over 16 weeks in which they developed progressively increasing serum IgG reactivity to SurVaxM peptide. **(B)** Survivin specific IgG response in one (representative) SurVaxM Phase II study patient. ELISA data shows patient serum responsivity to purified carrier protein (KLH), vaccine peptide (SurVaxM), wild type survivin or scrambled (non-specific) peptides at 1:500 dilution over the course of 30 weeks.



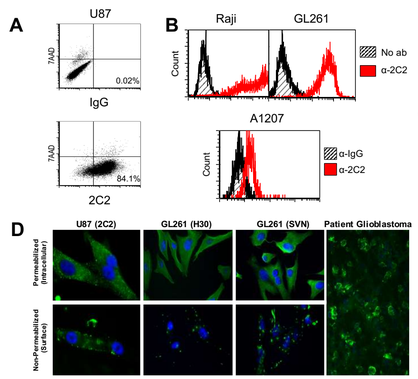


Figure 3. Detection of surface-survivin on cancer cells by flow cytometry and immunofluorescence. Flow analyses of cells labeled without fixation. **(A)** U87 human glioma cells stained with anti-survivin (2C2) and 7AAD as a viability marker. **(B)** Raji lymphoma, GL261 mouse glioma and A1207 human glioma stained with anti-survivin (2C2). **(C)** Anti-survivin (2C2) was pre-incubated with each of the indicated peptides prior to staining to show specificity through blocking. **(D)** Immunofluorescence staining of U87 and GL261 cells. Cells were fixed and permeabilized or directly probed with anti-survivin mAb’s. Staining of patient tumor (*right*) with anti-survivin mAb.

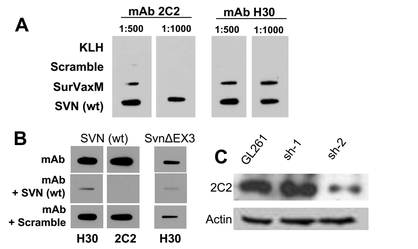
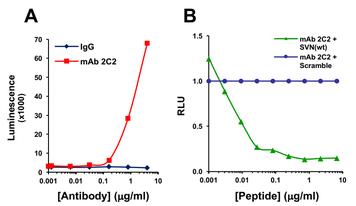


Figure 4. Antibody clones 2C2 and H30 detect survivin. (A) Slot blots showing 2C2 and H30 recognition of survivin peptides. Membrane-adhered wild-type (wt) survivin (aa 53-64), SurVaxM peptide, KLH, and scramble peptide were probed with mAb 2C2 or H30. **(B)** Slot blot showing detection of membrane-adhered SVN (wt) peptide or full-length SurvivinΔEX3 by mAb 2C2 or H30 when blocked with the indicated peptides. **(C)** Western blot of GL261 cell lysate and GL261 survivin-targeting shRNA transfectants.

Figure 5. Antibody clone 2C2 stimulates antibody-dependent cell-mediated cytotoxicity in Jurkat cells. ADCC was assessed using a reporter-based system in which effector (Jurkat) cells express luciferase upon engagement of mFcγRIV. **(A)** Dilutions of mouse anti-IgG or anti-survivin (2C2) were added to effector cells, and luminescence was measured. **(B)** Effector cells plus dilutions of Survivin peptide or a scramble peptide were incubated with 4 µg/mL anti-survivin mAb (2C2), and luminescence was measured. Data shows 2C2 normalized to scrambled (non-specific) peptide control; RLU = relative luminescence units.



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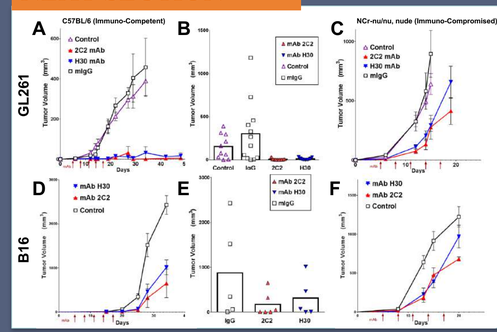

Figure 6 (Laft). Subcutaneous tumor models of GL261 (A-C) or B16-F1 (D-F). Immuno-competent C57BL/6 mice **(A-B, D-E)** or Immuno-Compromised nude mice **(C, D)** were compared. Injected cells were allowed to reach approximately 2 mm in tumor diameter before treating. Mice received 10 µg purified mAb or control IgG by i.p. injection every 3 days for 5 doses. Tumors were measured daily and volumes calculated (V=XY²/2). Data is shown as averaged tumor volumes +/- SEM, (GL261: *n*=8/group; B16: *n*=5/group; *p<0.0001).

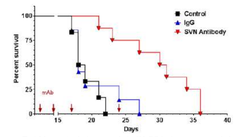

Figure 7 (Above). Intracranial GL261 tumor model showing efficacy of anti-survivin antibody against tumor growth. Starting on day 3, mice were i.p. injected with 10 µg of anti-survivin antibody or normal IgG as control every 7 days for 4 doses. Kaplan-Meier plot represents survival, (*n*=8/group; *=p<0.0001).

CONCLUSIONS & FUTURE DIRECTIONS

- Antibodies generated in response to SurVaxM immunization are highly cross-reactive to survivin and provide therapeutic benefit in immuno-competent mouse tumor models. These data demonstrate that administration of particular survivin antibodies are effective for reducing tumor volume and prolong survival.
- These antibodies retain some efficacy in immuno-compromised models, indicative of a direct T-cell independent effect.
- An anti-tumor response through anti-survivin targeted antibodies is unexpected as surface-accessible survivin expression has not yet been well described in the literature. Data presented here highlight possible avenues for investigation of mechanism(s) of action. Additional information can be found in AACR Abstract #2725 “Circulating CD9-GFAP-survivin exosomes during active specific immunotherapy, a potential biomarker for glioma.”
- A Phase II multi-center clinical study of SurVaxM in newly diagnosed glioblastoma is currently recruiting patients.
- The contribution of mAb to survival as part of the overall SurVaxM immune response (CD8, CD4, and mAb) in patients is currently being assessed.
- IND-enabling studies are in process with several SurVaxM mAb and mAb-derived pipeline agents.
- MimiVax, LLC is currently exploring developmental partnering of this asset.

REFERENCES & DISCLOSURES

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Disclosure Statements:

1. *MimiVax is a spin-off company of RPCI formed to develop and commercialize SurVaxM.*
2. *Michael Ciesielski & Robert Fenstermaker are co-founders and equity shareholders of MimiVax, LLC.*