Phase II Neoadjuvant and Immunologic Study of B7-H3 Targeting with Enoblituzumab in Localized Intermediate- and High-Risk Prostate Cancer

Eugene Shenderov[1], Karim Boudadi[1], Angelo DeMarzo[1], Mohamad E Allaf[1], Onur Ertunc[1], Igor Vidal[1], Carolyn Chapman[1], Hao Wang[1], Jim Vasselli[2], Jon Wigginton[2], Jan Davidson[2], Rehab Abdallah[1], Tanya O'Neal[1], Christian Pavlovich[3], Trinity Bivalacqua[3], Ashley E. Ross[3], Charles G. Drake [1,3], Drew Pardoll[1] & Emmanuel S. Antonarakis [1]

[1] The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins University, Baltimore, MD, USA; [2] MacroGenics, Inc., Rockville, MD, USA; [3] The James Buchanan Brady Urological Institute at Johns Hopkins University, Baltimore, MD, USA

BACKGROUND

- Prostate cancer (PCa) is the second-most-common cause of cancer-related death in men, killing approximately one in 50 American males.
- Immune-checkpoint blockade has resulted in unprecedented treatment advances in multiple tumor types, despite yielding modest results PCa.
- While CTLA-4 and PD-L1 are infrequently expressed in PCa, B7-H3 (another B7 superfamily member) is highly expressed in many PCas (**Fig. 1**), modulates anti-tumor immune responses, and is associated with worse prognosis (**Fig. 2**).

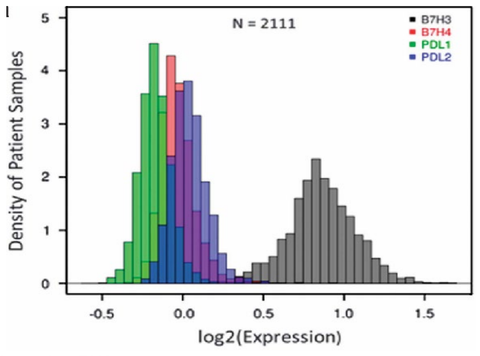


Figure 1: mRNA expression distributions of B7-H3, B7-H4, PD-L1 and PD-L2 from a radical prostatectomy cohort at Johns Hopkins.

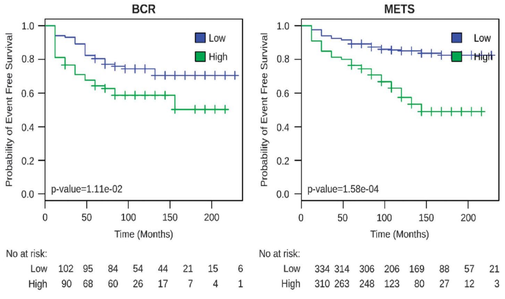


Figure 2. Survival curves for biochemical recurrence (BCR) and metastasis (METS) in a prostatectomy cohort (n=2111) stratified according to low and high B7-H3 mRNA expression.

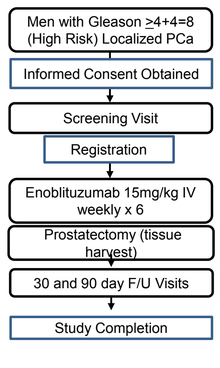
- Inhibiting B7-H3 is now clinically possible with the recent development of **Enoblituzumab** (MGA271, MacroGenics), a humanized Fc-optimized (for antibody-dependent cell-mediated cytotoxicity [ADCC]) monoclonal antibody that binds B7-H3.
- To date, approximately 180 patients have received Enoblituzumab monotherapy in phase I studies, with good tolerability.

STUDY HYPOTHESIS

- *Neoadjuvant Enoblituzumab treatment in patients with high-risk localized PCa will lead to partial pathological responses and reduced biochemical recurrence following prostatectomy, initially by modulating T cell immunity in the tumor microenvironment (TME) and also direct tumor killing via ADCC.*
- Additionally, the proposed immunologic analyses from these patients are expected to test the hypothesis that Enoblituzumab treatment enhances PCa-specific T cell responses systematically, and further, to identify additional immunologic targets for combinatorial immunotherapy.

STUDY DESIGN

- This is a single-center, single arm, phase 2 study evaluating the safety, anti-tumor effect, and immunogenicity of neoadjuvant Enoblituzumab (MGA271) given prior to radical prostatectomy in men with intermediate and high-risk localized prostate cancer (Gleason sum 7-10)
- Eligible patients (n=32) will receive Enoblituzumab at a dose of 15mg/kg IV given weekly for 6 doses beginning 50 days prior to radical prostatectomy
- 14 days after the last dose of Enoblituzumab, prostate glands will be harvested at the time of radical prostatectomy, and prostate tissue will be examined for the secondary endpoints
- Follow-up evaluation for adverse events will occur 30 days and 90 days after surgery. Patients will then be followed by their urologists according to standard institutional practices, but will require PSA evaluations every 3 (±1) months during year 1 and every 6 (±2) months during years 2-3 (**Fig. 3**).



- Key Inclusion Criteria:
- Histologically confirmed prostate adenocarcinoma; clinical stage T1c–T3b, N0, M0; Gleason sum 7-10; at least 2 positive cores; prior decision to undergo radical prostatectomy; adult male >18 years of age; ECOG performance status 0-1, or Karnofsky score ≥ 70%

- Key Exclusion Criteria:
- Presence of known lymph node involvement or distant metastases; prior radiation, hormones, biologics, or chemotherapy for prostate cancer; prior immunotherapy/vaccine therapy for prostate cancer; prior use of experimental agents for prostate cancer; concomitant treatment with hormonal therapy or 5a-reductase inhibitors; history of autoimmune disease requiring systemic immunosuppression

Figure 3. Study schema for the neoadjuvant Enoblituzumab clinical trial (**NCT02923180**).

SPECIFIC AIMS

(1) To investigate whether Enoblituzumab mediated B7-H3 inhibition is safe, effective and immunologically active in the pre-surgical PCa setting by conducting a phase II neoadjuvant clinical trial in 32 men with high-risk localized PCa scheduled for prostatectomy

(2) To determine whether Enoblituzumab results in pathologic anti-tumor responses by evaluating tumor cell apoptosis and TME T cell infiltrates (CD4+/8+ T cell density, Treg density, NK density, and CD8+/Treg and CD4+Tconv/Treg ratios) pre- and post-treatment

(3) To interrogate mutation-associated neoantigen-specific T cell responses induced by anti–B7-H3 therapy, analyze targetable immune-checkpoints adaptively-induced upon Enoblituzumab treatment, as well as elucidate the repertoire and gene-expression profiles of tumor-specific tumor-infiltrating T cells (TILs) utilizing multi-parameter flow cytometry and RNAseq. This first-in-field translational study of Enoblituzumab in PCa will allow concurrent exploration of its clinical efficacy and anti-tumor immunity.

PRELIMINARY RESULTS

Enoblituzumab binds B7-H3 with high affinity and specificity

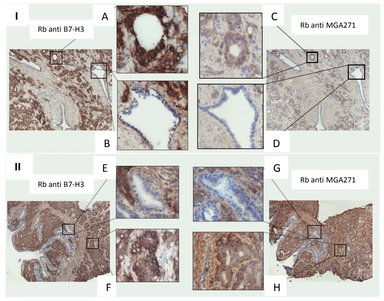


Figure 4. Prostatectomy immunohistochemistry staining from two patients (**I** and **II**) following neoadjuvant Enoblituzumab treatment. Small malignant glands lined by enlarged atypical epithelial cells show clear membrane staining by both anti-B7-H3 (**A** and **F**) and anti-Enoblituzumab (anti-MGA271, **C** and **H**). Adjacent non-malignant prostatic ducts show relatively negative membrane staining (**B+E** and **D+G**).

CD8+ T Cell Infiltrates Detected in Neoadjuvant Enoblituzumab Treated Prostatectomies

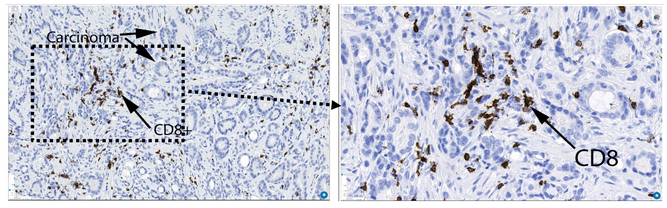


Figure 5. Prostatectomy immunohistochemistry (IHC) staining from a patient following neoadjuvant Enoblituzumab treatment. Shown are CD8+ T cell infiltrates (arrows) which are in close proximity to atypical malignant glands (arrows).

CD8+ T cell quantitation in the Enoblituzumab-treated prostatectomy samples indicates a statistically significant increase in infiltrate compared to age- and stage-matched untreated prostatectomy controls

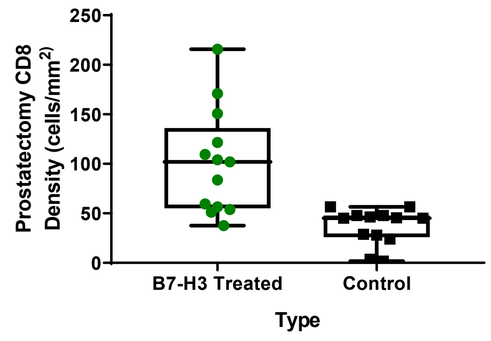


Figure 6. CD8 T cell density in prostatectomy controls versus neoadjuvant Enoblituzumab-treated patients. All prostatectomy slides from the first 13 patients treated with neoadjuvant Enoblituzumab were retrieved from the pathology archives. A representative tumor block containing the highest-grade lesion was obtained, sectioned and stained for CD8 T cells using a validated antibody protocol on a Ventana Discovery Ultra system. Slides were counterstained with hematoxylin and scanned using the Aperio ScanScope scanner. Aperio Spectrum image analysis software was used for quantification. For controls, prostate glands from untreated patients matched by Gleason grade, pathological stage and age were used. As shown, CD8 T cell density was significantly higher among Enoblituzumab-treated patients compared to untreated control patients (median 98 *vs* 46, *P*=0.0007).

KEY STUDY ENDPOINTS

Primary endpoints:
- Frequency, type, and severity of adverse events
- Estimation of clinical benefit based on the PSA_0 response rate (PSA <0.1 ng/mL) at 12 months after radical prostatectomy, as well as time to PSA recurrence and pathologic response

Key secondary endpoints:
- Quantification of Enoblituzumab-induced tumor cell death (via direct ADCC or indirectly via T cell killing) using TUNEL staining and cleaved Caspase 3 staining
- To assess the immune response to Enoblituzumab using quantification of CD8 T cell infiltration into the tumor/ peri-tumoral areas, determining the effect of Enoblituzumab treatment on the CD8/Treg ratio, and quantifying the extent of PD-L1+ cell density in the prostate from harvested prostate glands of treated patients

Correlative endpoints:
- To quantify B7-H3 expression in pre-treatment and post-treatment tumor tissue, and associated tumor cell apoptosis
- To quantify induced checkpoint ligand and receptor expression (*i.e.* treatment-induced adaptive resistance, focusing on PD-L1, PD-1, TIGIT, PVR-Ig, VISTA, LAG3 and TIM3, all of which are targets for existing clinical antibodies) in pre- and post-treatment tumor tissue
- To determine Fc receptor genotype (CD16A, CD32A, CD32B), which could affect Enoblituzumab's ADCC activity as it does with Rituximab
- Elucidate the expression profile of pre- and post-treatment tumor tissue using the NanoString immunopanel
- To analyze the tumor-specific repertoire using TCRseq-based techniques, testing the hypothesis that successful anti-tumor responses modulate the TCR repertoire in peripheral and tumor-infiltrating lymphocytes and assessing relative responses to mutation-associated neoantigens (MANAs) vs PCa tumor-associated antigens (TAAs).

SUMMARY

This study aims to understand the impact of B7-H3 targeting/blockade on PSA recurrence following prostatectomy, impact on the prostate gland tumor microenvironment (TME), and assess whether (like PD-L1 status) B7-H3 IHC staining can be used to predict response or resistance to B7-H3–targeted therapies.

REFERENCES

Benzon et al. Prostate Cancer Prostatic Dis. 2016 Nov 1.
Kim et al. EBioMedicine. 2016 May;7:85-93.
Parker et al. Int J Radiat Oncol Biol Phys 2011 Apr 1;79(5):1343-9.
Powderly J, et al. SITC abstract 2015.
Roth et al. Cancer Res 2007 Aug 15;67(16):7893-900.

ACKNOWLEDGEMENTS

Study sponsors: Emmanuel Antonarakis, M.D. (PI); MacroGenics, Inc., Rockville, MD

Study contacts: Eugene.Shenderov@jhmi.edu (presenting author), eantona1@jhmi.edu (corresponding author)

CONQUER CANCER™ THE ASCO FOUNDATION YOUNG INVESTIGATOR AWARD RECIPIENT