Opening the Floodgates to T Cells – Biomarkers for the Combination of Bevacizumab and Atezolizumab in RCC

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The goal of CIT approaches is to promote anti-tumor immunity

Inflamed

- Respond favorably to checkpoint inhibition
- TILs
- CD8+ T cells
- PD-L1 expression
- Genomic instability
- Pre-existing immunity

Non-inflamed

- How do you convert these tumors to inflamed tumor?
Is VEGF inhibition synergistic with anti-PD-L1?

PRIMING AND ACTIVATION

CANCER ANTIGEN PRESENTATION

RELEASE OF CANCER CELL ANTIGENS

TRAFFICKING OF T CELLS TO TUMORS

INfiltration of T CELLS INTO TUMORS

RECOGNITION OF CANCER CELLS BY T CELLS

KILLING OF CANCER CELLS

Atezolizumab (anti-PD-L1)


Bevacizumab (anti-VEGF)

- Can Res 2010, 70; 6171
Overview of GP28328 Ph1b Trial Design

- Six arms (A-F), open label study
  - Arms A, B → Bev +/- chemo in solid tumors
- 1° objectives: safety, tolerability, DLT and MTD

Biopsy and blood collection schedule

Flow cytometry, plasma cytokines, Fluidigm, Nanostring, IHC, TCR Sequencing
Efficacy: Tumor burden over time in mRCC patients

- 40% Overall Response Rate (ORR)
  - Historical response rates with atezolizumab and bevacizumab are ~15% and ~9%, respectively
- Combination is well-tolerated
- 6 of 10 patients still on study after 15 mos

Bendell, Jones, Mier, Sznol, McDermott

Investigator-assessed unconfirmed response per RECIST v1.1.
IC, immune cells; IHC 3: ≥ 10% tumor-infiltrating ICs positive for PD-L1; IHC 2: ≥ 5% and < 10% tumor ICs positive for PD-L1; IHC 1: ≥ 1% and < 5% tumor ICs positive for PD-L1; IHC 0: < 1% tumor ICs positive for PD-L1.
Efficacy evaluable patients dosed by April 7, 2014, who had at least 1 scan; data cutoff July 7, 2014.
Approaches to study human tumor immune biology

**iCHIP**
- High throughput and comprehensive evaluation of tumor and immune genes

**TUMOR**
- Spatial assessment of CD8 in response to treatment
- Dx grade assays for assessment of target expression

**IMAGING**
- Allow for evaluation of multiple immune cell subsets, endothelial cells, and tumor cells
- Enables spatial assessment of TILs

**BLOOD**
- Cytokines/chemokines
- Circulating Immune Cell Subsets
  - Mutational burden
  - TCR sequencing
Increases in CD8\(^+\) T cells are observed with treatments in RCC

Patient 3, Female, 62 years old

- 83\% (5/6) of bev + atezo RCC patients had increases in tumor CD8\(^+\) T cells
- 11\% (1/9) of RCC patients had increased tumor CD8\(^+\) T cells following monotherapy atezo (PCD4989g)
Endothelial and immune marker changes are observed with treatments.

Patient 3, Female, 62 years old

MHC I

Bendell, SCRI
Downregulation of vascular gene signature and upregulation of immune effector gene signatures in the tumor with Bev+Atezo

Vascular gene signature - ANGPT2, CD34, DLL4, EGFL7 and ESM1
CD8 T effector gene signature - CD8A, CD8B, EOMES, GZMA, GZMB, IFNG, and PRF1
Th1 chemokine signature - CXCL10, CXCL11, CXCL13, and CXCL9
NK cell gene signature - GZMB, KLRD1, and SLAMF7
Why are CD8$^+$ TILs increased in the tumor following Bev+Atezo?

Possible explanations:

• CD8$^+$ T cells are proliferating in the tumor

• CD8$^+$ T cells are recruited and/or allowed to infiltrate the tumor
  
  – Anti-VEGF-induced changes in the vasculature allow CD8$^+$ T cells to invade tumor tissue

  – CD8$^+$ T cells are recruited to the tumor by “stressed/inflamed” endothelial cells after anti-VEGF therapy
Increases in proliferating CD8+ T cells are detected on-treatment

Patient 1

CD8/Ki67

Pre-treatment

Post Bev

Post Bev+Atezo

Patient 5

Patient 6
CD8+ TIL increase does not appear to be due to enhanced proliferation within the tumor.

Greater density of CD8+ T cells post-combination, but ratio of Ki67+ proliferating and Ki67- non-proliferating cells unchanged.
Why are CD8$^+$ TILs increased in the tumor following Bev+Atezo?

**Possible explanations:**

- CD8$^+$ T cells are proliferating in the tumor

- CD8$^+$ T cells are recruited and/or allowed to infiltrate the tumor
  
  - *Anti-VEGF-induced changes in the vasculature allow CD8$^+$ T cells to invade tumor tissue*
  
  - *CD8$^+$ T cells are recruited to the tumor by “stressed/inflamed” endothelial cells after anti-VEGF therapy*
Infiltrating cells are located near unstable vessels on-treatment.

CD8\(^+\) T cells and CD163\(^+\) macrophages are located near immature vessels (\(\alpha\)SMA\(^-\))
Expression of fractalkine (CX3CL1) and its receptor (CX3CR1) are elevated with Bev + Atezo

- CX3CL1 is a potent chemoattractant expressed on the surface of endothelial cells
- CX3CR1 is highly expressed on armed CD8+ T effectors, NK cells, and macrophages
T cell receptor (TCR) sequencing identifies individual T cell clones

$10^{14}$ TCRs
$\sim 5M$ clones

**Diversity** – # unique clones

**Clonality** – relative abundance

**Public clones** – shared clones (disease-specific)

Wang et. al., Cancer Res 2012
Changes in TIL composition after combination signifies an evolving anti-tumor T cell response

This patient had a 5.7-fold increase in intratumoral CD8+ T cells on-treatment
Summary and Conclusions

- Combination of bevacizumab+atezolizumab is active with a 40% ORR and prolonged duration of response.

- Anti-VEGF treatment alone modulates the tumor immune microenvironment via increased Th1 signaling, fractalkine expression and expression of MHC Class I on tumor cells.

- Increased proliferating CD8+ T-cell infiltration post bevacizumab+atezolizumab observed in close proximity to unstable vessels but not stable vessels. This is co-incident with contextual macrophage expression around unstable but not stable vessels.

- The T cell repertoire in the tumor is changed on-treatment with bev alone and bev+atezo, which may provide support for a selective trafficking mechanism. It is also possible that the infiltration is non-biased and there is retention of antigen-specific T cells in the tumor.

- Phase II and III studies are currently ongoing
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Genentech/Roche investigators and patients
The Cancer-Immunity Cycle

1. Release of cancer cell antigens
2. Cancer antigen presentation
3. Priming and activation
4. Trafficking of T cells to tumors
5. Infiltration of T cells into tumors
6. Recognition of cancer cells by T cells
7. Killing of cancer cells