**Introduction**

TGF-β and the immune system

- The TGF-β pathway can promote cancer progression and immune resistance. TGF-β signals to tumor cells, which can induce angiogenesis, stromal, and epithelial-mesenchymal transition (EMT). Several studies have shown that TGF-β and its receptors have a role in controlling immune cells' recruitment and activity. The *Tumor microenvironment* may influence tumor cell behavior, and TGF-β production can be affected by immune cell infiltration.

- Molecular tricks in tumor cells and regulatory T cells have demonstrated frequent dysregulation of TGF-β signaling and suppression of the T-cell mediated immune response in tumor examples.

- TGF-β activity in the TME might decrease the efficacy of, or even promote resistance to, antitumor therapies, including anti–PD-1/L1 therapies.

- Thus, TGF-β activity in the TME might be a novel treatment approach.

- Additionally, different tumor immune phenotypes, each characterized by the level of immune cells infiltrating the tumor area, have been shown to affect responses to different immunotherapies across tumor types.

**Methods**

- Bintrafusp alfa is a first-in-class bifunctional fusion protein composed of a nontoxic subunit (C-terminal domain of TGF-βRII) that blocks TGF-β signaling and an immunostimulatory subunit (PD-L1 and PD-L2 negatively regulated) to enhance antitumor activity.

- Gene expression analysis was performed using RNA sequencing data as previously described. A pathologist who was masked to the response data scored the scanned images. Tumors were categorized into 3 immune phenotypes using an exploratory classification system:

  - **Immune-desert**
    - A subset of patients in the NSCLC cohort (n=40) received bintrafusp alfa for up to 2 years (Q2W). The primary objectives were to evaluate response rates and safety in the NSCLC phase I/II studies.

  - **Immune-excluded**
    - Immune-excluded tumors demonstrate a lower level of immune cells, such as lymphocytes, in the tumor stroma area.

  - **Inflamed**
    - The inflamed phenotype was observed in patients with immune-desert tumors.

- **Results**

  - Across different tumor types, patients with immune-desert tumors exhibited a lower level of immune genes, such as **GZMB** and **GZMA**, compared to inflamed tumors.

  - **Immune-excluded vs Inflamed**
    - Immune-desert tumors (4/5; 80.0%) and ESCC (3/3; 100%) were more common in tumors with an immune-excluded phenotype, whereas immune-desert tumors (163/247; 66.0%) and inflamed tumors (80/247; 32.5%) were more common in tumors with an inflamed phenotype.

- **Conclusions**

  - While the distribution of immune phenotypes varied by tumor type and clinical setting, patients with immune-excluded tumors were more common in tumors with both TGF-β signaling and PD-(L)1 activity.

  - The inflamed phenotype was more common in tumors with TGF-β signaling and PD-(L)1 activity, indicating a potential role for combination therapies.

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