**Introduction**

Monocytes are crucial for T cell stimulation via the PD-L1 pathway, revealing an ambiguous role for TNF-α and PD-L1 with anti-PD-L1 therapy, only the lung TME infiltrated monocytes: produce and respond to increased TNF-α levels, increase their levels of LAG-3, TIM-3, SIRP-α and VISTA.

**Hypothesis**

**Do PD-(L)1+ myeloid cells hamper effective anti-PD-L1 therapy?**

**Conclusions**

1. Upon anti-PD-L1 therapy, only the lung TME infiltrated monocytes:
   - produce and respond to increased TNF-α levels
   - increase their levels of LAG-3, TIM-3, SIRP-α and VISTA
2. TNF-α is involved in anti-PD-L1 therapy-mediated checkpoints
3. TNF-α and PD-L1 co-blockade has no increased therapeutic benefit
4. Monocytes are crucial for T cell stimulation via the combination of anti-PD-L1 with anti-SIRP-α or anti-LAG3

**Results**

1. Anti-PD-L1 therapy has no therapeutic benefit despite elevated PD-L1 expression levels in lungTME

**Characterization of the lung tumor microenvironment upon anti-PD-L1 therapy reveals an ambiguous role for TNF-α**

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**Methods**

- In vivo set-up
  - In vivo BLI and 3D whole tissue imaging
  - To follow tumor progression

- Multiplex flow cytometry
  - To characterize 11 myeloid subsets
  - PACS
  - To isolate macrophages and monocytes

- Use in T cell killing assay
  - Monitor immune profile via flow cytometry
  - Use in T cell killing assay

**5. TNF-α fortifies the upregulation of checkpoint molecules on anti-PD-L1 treated monocytes**

- Anti-PD-L1 therapy abolishes the rise in MHC-IIP+ TAMs and monocytes upon LLC progression
- Anti-PD-L1 therapy results in a monocyte-specific TNF-α response
- Anti-PD-L1 - PD-L1 co-blockade has no increased therapeutic benefit
- TNF-α + PD-L1 + anti-PD-L1 - anti-TIM3

**6. Monocytes play a key role in the CTL-stimulating potential of ICB combination therapy**

- Anti-PD-L1 - anti-TIM3
- Combined therapies
  - No T cells
  - anti-PD-L1
  - anti-VISTA
  - anti-PD-L1 + anti-VISTA
  - anti-LAG-3
  - anti-PD-L1 + anti-LAG-3
  - anti-SIRPA
  - anti-PD-L1 + anti-SIRPA
  - anti-TIM3
  - anti-PD-L1 + anti-TIM3