LCMC3: Immune Cell Subtypes Predict Pathologic Response After Neoadjuvant Atezolizumab in Resectable NSCLC

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## Disclosures

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**LCMC3 study design**

**Primary endpoint:**
- MPR (≤10% viable tumor cells)

**Exploratory endpoints:**
- Biomarkers: flow cytometry, scRNAseq, bulk RNAseq, TCRseq

- LCMC3 is the largest reported study of anti–PD-L1 neoadjuvant therapy conducted to date (n=181)
- We explored whether the peripheral blood immunophenotype assessed via 10-color 60-marker flow cytometry and the tumor microenvironment (TME) assessed via RNAseq would be predictive of MPR
  - Comprehensive immunophenotyping via 10-color 60-marker IMMUNOME flow cytometry of peripheral blood at baseline
  - Tumor scRNAseq data (n=13) and tumor bulk RNAseq data from pre- (n=56) and post-treatment (n=44) samples

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**CT, PET-CT**

1. **Resectable, untreated, unselected stage IB-IIIA, select IIIB NSCLC N=181**
2. **Atezolizumab (2 cycles)**
   - Tumor biopsy
   - Lymph nodes
   - Blood
3. **Surgical resection**
   - Tumor
   - Lymph nodes, normal lung
   - Blood
4. **30-day post-surgery visit**
   - Blood
5. **Surveillance + optional adjuvant atezolizumab (12 months)**
   - Blood, q3mo
   - Progression biopsy

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CT, computed tomography; MPR, major pathological response; NSCLC, non-small cell lung cancer; PET, positron emission tomography; q3mo, every 3 months; RNAseq, RNA sequencing; scRNAseq, single-cell RNA sequencing; SOC, standard of care; TCRseq, T-cell receptor sequencing.

*a Mandatory. NCT02927301.*

Baseline peripheral blood immunophenotypes predict MPR

ROC curves for immune cell subsets (n=115)

- IMMUNOME flow cytometry data from pre-treatment peripheral blood samples (n=115) were used to build an immune cell model predictive of MPR.
- The algorithm was informed by 13 samples each from patients with ≥88% viable tumor cells and patients with ≤20% viable tumor cells at surgery.
- Pre-treatment peripheral blood samples were placed into training or testing sets and analyzed using an approach based on generalized additive models and regularized regression (LASSO). Immune cell subsets detected in fewer than 50% of samples were excluded.
- 13 immune cell subsets in the baseline peripheral blood sample predicted MPR, including NK-cell and NK-like T-cell subtypes expressing ILT2 and NKG2A.

AUC, area under the curve; LASSO, least absolute shrinkage and selection operator; MPR, major pathological response; NK, natural killer; ROC, receiver operating curve.

- **Positively associated with MPR**
  - Non-T/non-NK cells: ILT2"NKG2A"CD63"CD3"CD158e1"CD158b"CD56"KIR2DL1"CD16" CD94"NKG2D"CD3"CD56"CD117"CD127"CD161"CD162
  - NK cell: NKG2A"HLA-DR"CD69"CD3"CD158e1"CD158b"CD56"KIR2DL1"CD162

- **Negatively associated with MPR**
  - NK cells: CD16"CD336"CD3"CD244"CD335"NKG2D"CD56"CD161"CD337+
  - ILT2"NKG2A"CD63"CD3"CD158e1"CD158b"CD56"KIR2DL1"CD162
  - NK-like T cells: NKG2A"HLA-DR"CD69"CD3"CD158e1"CD158b"CD56"KIR2DL1"CD162
  - ILT2"NKG2A"CD63"CD3"CD158e1"CD158b"CD56"KIR2DL1"CD162
  - γ/δ T cells: vδ1/2α/β"CD19"CD56"CD16"CD13/14"CD4"CD3"CD8+
  - vδ1/2α/β"CD19"CD56"CD16"CD13/14"CD4"CD3"CD8+ 
  - vδ1/2α/β"CD19"CD56"CD16"CD13/14"CD4"CD3"CD8+

- **Naive T cell**: CD62"CD27"CD56/16"CD45RO"CCR7"CD45RA"CD4"CD3"CD8+

AUC, area under the curve; LASSO, least absolute shrinkage and selection operator; MPR, major pathological response; NK, natural killer; ROC, receiver operating curve.

- a Non-squamous vs squamous. b N1/N2 vs N0.
Prediction of MPR via immune cells in pre-treatment peripheral blood

- The probability of achieving MPR was calculated based on the immunophenotype (IMMUNOME) of each patient using the LASSO model.

- As a second testing cohort, patients with PD who were not part of the LASSO model were evaluated for their probability of achieving MPR based on their pre-treatment blood IMMUNOME.

- The probability of achieving MPR using the predictive IMMUNOME model was low in the PD cohort (second testing cohort).

LASSO, least absolute shrinkage and selection operator; MPR, major pathological response; PD, progressive disease.

- Sample was drawn before treatment was started. 9 of 11 patients with PD did not undergo surgery. This population was not included in the training and testing cohorts.

TME analysis showed innate immune markers were associated with MPR

- TME scRNAseq data showed high ILT2 expression on macrophages, monocytes and DCs, and high PD-L1 expression on DCs; NK2G2A and KIR2DL1 were mostly expressed on NK cells
- Bulk RNAseq data at baseline revealed significantly more ILT2 expression in MPR patients and a linear correlation between ILT2 and PD-L1 expression in the TME, suggesting a co-expression of ILT2 and PD-L1 on the same cells
- By bulk RNAseq, PD-L1 and ILT2 expression were both positively associated with the abundance of DCs and CD8+ T cells

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* Dot size represents percentage of NK cells in the group expressing the gene. b Color represents scaled average normalized expression.
We show for the first time in patients with resectable NSCLC treated with neoadjuvant atezolizumab that MPR may be predicted by innate immune markers assessed via 10-color 60-marker IMMUNOME flow cytometry in pre-treatment peripheral blood.

• Results were validated with 2 testing cohorts in LCMC3

• Innate immune cells including ILT2- and NKG2A-expressing NK cells and NK-like T cells in the peripheral blood were associated with the anti-cancer immune response to treatment with neoadjuvant atezolizumab

• Tumor RNAseq data revealed a positive association of ILT2 expression with MPR, which is mostly expressed on dendritic cells, macrophages and monocytes and linearly associated with PD-L1 expression, suggesting co-expression of ILT2 and PD-L1 on the same cells

• These data could inform novel therapeutic approaches such as combination treatments with immunotherapies and inhibitors of other checkpoints, including NKG2A/HLA-E and ILT2/HLA-G

Conclusions

MPR, major pathological response; NSCLC, non-small cell lung cancer; RNAseq, RNA sequencing; NK, natural killer.
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