NKG2A immune checkpoint blockade potentiates cetuximab induced ADCC in head and neck cancer preclinical model

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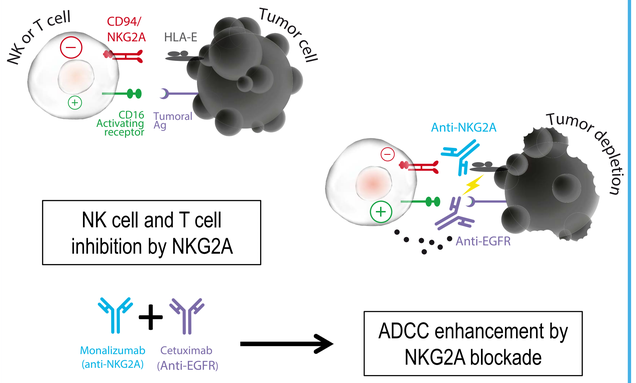
Background

Monalizumab (IPH2201) is a first-in-class humanized IgG_4 targeting NKG2A (Natural Killer Group 2A), which is expressed as a heterodimer with CD94 on subsets of NK cells, γδ T cells and tumor infiltrating $CD8^+$ T cells. This inhibitory receptor binds to HLA-E (Human Leukocytes Antigen-E) molecules which are frequently upregulated on cancer cells providing a negative regulatory signal to tumor-infiltrating lymphocytes (TILs). Monalizumab blocks binding of CD94/NKG2A to HLA-E, reducing inhibitory signaling and thereby unleashing NK and T cell responses.

High expression of EGFR (epidermal growth factor receptor) occurs in most epithelial malignancies and particularly in squamous cell carcinoma of the head and neck (SCCHN) and is associated with poor prognosis. The anti-EGFR monoclonal antibody cetuximab (Ctx) is thought to act through blocking oncogenic signaling and by inducing Fcγ receptor-mediated antibody dependent cell cytotoxicity (ADCC) which involves human NK cells.

Here, we investigated ***ex vivo*** and ***in vitro*** the rationale of combining monalizumab with Ctx in the treatment of head and neck cancers.

Mechanism of Action

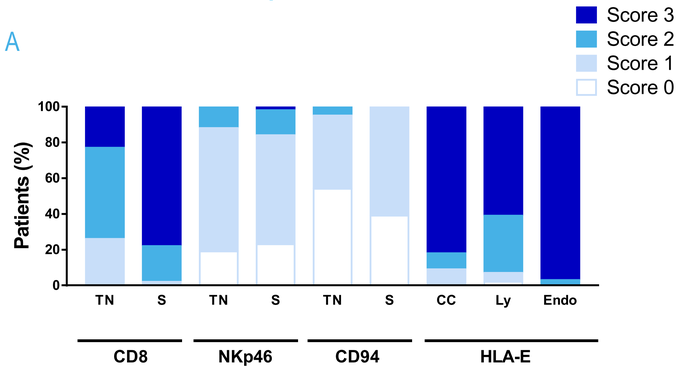


NK cell and T cell inhibition by NKG2A

ADCC enhancement by NKG2A blockade

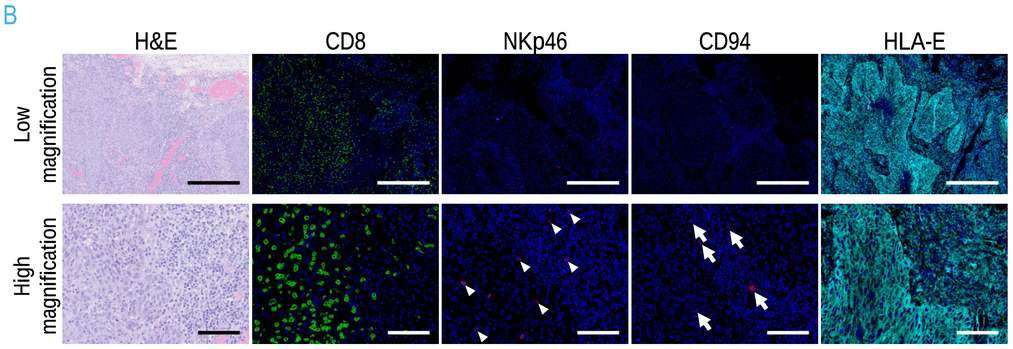
SCCHN tumor are infiltrated by $CD8^+$ T, NK and $CD94^+$ immune cells and cancer cells express HLA-E

A



A/ Semi-quantitative analysis of $CD8^+$, $NKp46^+$, $CD94^+$ cells and HLA-E expression on human SCCHN. FFPE samples (n=65). $CD8^+$, $NKp46^+$ and $CD94^+$ cells were quantified in two different tumor regions: stroma (S) and tumor nests (TN) and HLA-E expression was assessed on cancer cells (CC), lymphocytes (Ly) and endothelial cells (Endo). Score 0= no positive cell; Score 1= between 1 and 33% positive cells; Score 2= between 34 and 66% positive cells and Score 3 ≥66% positive cells. For each marker stacked bars represent the percentage of patients with score 0, 1, 2 or 3.

B



B/ Representative example of SCCHN highly infiltrated by CD8, NKp46 and CD94 positive cells and having high HLA-E expression on tumor cells. White arrowheads indicate $NKp46^+$ cells and white arrows indicate $CD94^+$ cells. Scale bars correspond to 500µm (upper panel) or 100µm (lower panel). *H&E, hematoxylin and eosin.*

NKG2A blockade enhances cetuximab-mediated ADCC in a dose dependent manner

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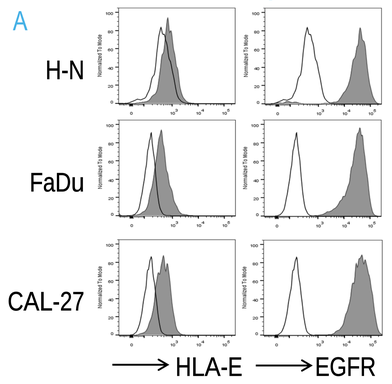
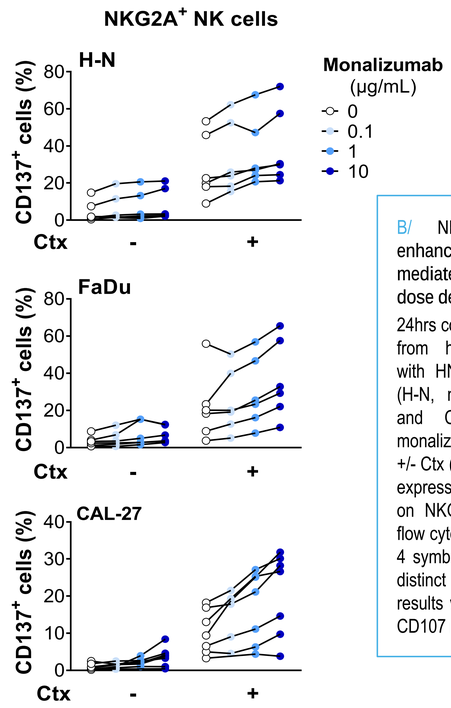


A/ HLA-E and EGFR expression in HN tumor cell lines.

Cell lines were labeled with either anti-HLA-E Ab (clone 3D12), Ctx, or unrelated IgG_1 as an isotype-matched control. Open histogram: isotype control; filled histogram, PE-conjugated anti-HLA-E or Ctx revealed with PE-conjugated secondary anti-huIgG.

B



B/ NKG2A blockade enhances cetuximab mediated ADCC in a dose dependent manner.

24hrs co-cultures of PBMC from healthy volunteers with HN tumor cell lines (H-N, n= 6; FaDu, n=6 and CAL-27, n=7) + monalizumab dose-range +/- Ctx (0.1µg/mL). CD137 expression was analyzed on $NKG2A^+$ NK cells by flow cytometry. Each set of 4 symbols represents one distinct donor. Similar results were obtained with CD107 read-out.

SCCHN is one of the tumor types with the highest NK cell density

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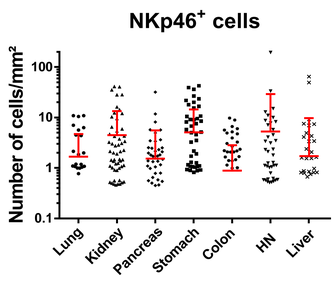

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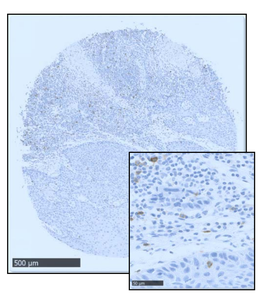
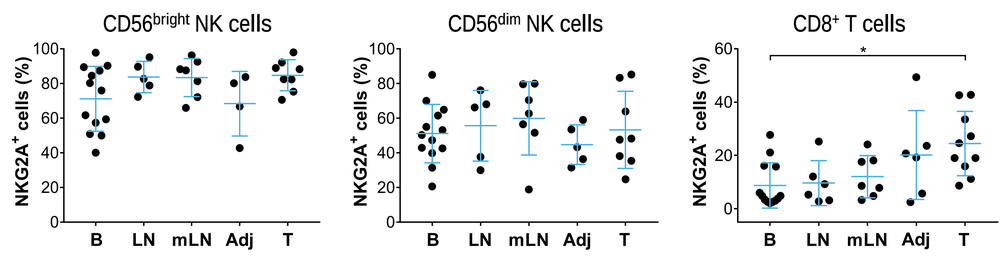


A/ Quantitative analysis of $NKp46^+$ cells (NK cells) using Halo (Indicalabs) in lung (n=45), kidney (n=75), pancreas (n=77), stomach (n=76), colon (n=100), HN (n=68) and liver tumors (n=106). Formalin-fixed paraffin-embedded (FFPE) tissue microarrays were stained by IHC using anti-NKp46 (clone 8E5B). Scatter plots of the densities of $NKp46^+$ cells (nb of cells/mm²). Red bars indicate the mean (±SD) of the cell densities.
B/ Representative example of SCCHN highly infiltrated by $NKp46^+$ cells.

NKG2A is expressed on tumor infiltrating $CD8^+$ T cells and NK cell subsets in HN patients



Expression of NKG2A was analyzed on $CD56^{bright}$ NK cells (left), $CD56^{dim}$ NK cells (middle) and $CD3^+CD8^+$ T cells (right) from peripheral blood (B), lymph node (LN), metastatic lymph node (mLN), adjacent tissue (adj) and tumor (T) by flow cytometry. 15 patients were analyzed; the number of organs available varies from one patient to another. Horizontal bars are mean ± SD. Kruskall Wallis followed by Dunn's test.

Conclusions

- SCCHN are infiltrated by NK and $CD8^+$ T cells expressing CD94/NKG2A.
- HN tumor cells express HLA-E.
- NKG2A blockade enhances cetuximab-mediated ADCC towards HN tumor cell lines in a dose-dependent manner.
- Altogether, these data support the rationale for investigating monalizumab in SCCHN patients and in combination with cetuximab in clinical trials (NCT02643550; see poster CT158).