Effects of anti-DLL4 treatment on non-small cell lung cancer (NSCLC) human xenograft tumors

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ABSTRACT

Background: Non-small cell lung cancer (NSCLC) accounts for the vast majority of lung cancers, the leading cause of cancer-related death worldwide. NSCLC tumors often harbor mutations in epidermal growth factor receptor (EGFR) and KRAS genes which are important for cancer stem cell (CSC) survival. Cemizumab (OML-2191) is a humanized IgG2 anti-DLL4 antibody currently being tested in Phase I clinical trials targeting cancer stem cells and promoting anti-angiogenesis by inhibiting cancer stem cells and promoting tumor vasculature regression. Here we show results from NSCLC-PDX models.

Methods and Findings: Anti-DLL4 treatment was tested in a series of NSCLC-PDX models. Because DLL4 inhibition has been shown to have effects on tumor as well as the vasculature, the combination of OML-2191 (inhibiting human DLL4) and 21M18 (antibody targeting mouse DLL4) treatment in the PDX models was used to model demecizumab treatment in humans. Treatment with anti-DLL4 in combination with a small molecule Notch pathway inhibitor, or with demecizumab combined with a small molecule Notch inhibitor, showed a decrease in the frequency of tumor initiating cells following treatment with anti-DLL4 and chemotherapy. Gene-expression analyses of tumors provided insights into mechanisms of action and gene expression changes between tumor samples treated with and without DLL4 inhibition.

Conclusions: Anti-DLL4 treatment is a panel of NSCLC-PDX tumor models in vivo showed inhibition of tumor growth and a decrease in the frequency of tumor-initiating cells. Mechanism of action and gene-expression analyses of these models treated with and without DLL4 will be presented. These findings provide additional evidence supporting DLL4 as a therapeutic target.

MATERIALS AND METHODS

The recombinant antibodies denoted (OML-2191) and OML-2189 (anti-DLL4) and OML-2193 (anti-DLL4) were generated at OncMed Pharmaceuticals and dosed together (as DLL4) in all experiments. The NSCLC tumor biopsies used to establish the tumor xenografts were obtained from patients diagnosed with non-small cell lung cancer. Pharmacological inhibitors were purchased from Selleck Chemicals Company (SelleckChem). Monoprep (DDFR) or NCI-H460, and Caliocytes (human T cell lines) were obtained from Dr. C. L. Murriel (University of Colorado). OML-2191 was dosed subcutaneously in FLH-1066 and was dissolved in PBS. Tumors were allowed to grow to 115-120 mm3 and were randomized into treatment groups. Experimental groups included treatment with control antibodies (115-120 mm3), OML-2191 (20 mg/kg), standard of care chemotherapy (cisplatin 35-50 mg/kg, or paclitaxel 10-15 mg/kg, or carboplatin 25-50 mg/kg), in combination with antibodies and chemotherapy. The specificity of RNA and protein expression analysis, tumors were stained with RFP and WGA (live). The quality of RNA and protein expression analysis, tumors were stained with RFP and WGA (live).

The data were analyzed using the genes expression analysis software package Genes (GSEA) (v. 2.2.6) and MSigDB c3.h.all which was used for downstream analysis of gene sets. The number of experiments was performed in biological triplicate.

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REFERENCES


IN VIVO TUMOR GROWTH IS INHIBITED WITH ANTI-DLL4 AND COMBINATION TREATMENT IN NSCLC PDX TUMORS

Notch target genes including HEY1 are down-regulated with anti-DLL4

Stem cell gene sets are down-regulated with anti-DLL4

ANTITUMORIC GENE SIGNATURES ARE UP-REGULATED IN STROMA FROM ANTI-DLL4 TREATED NSCLC PDX TUMORS

Previously defined DLL4 gene signature is up-regulated in NSCLC stroma

VASCULARITY-RELATED GENES ARE UP-REGULATED IN STROMA FROM ANTI-DLL4 TREATED NSCLC PDX TUMORS

Vascular-related genes including previously observed Chai5 and Apol are up-regulated in treated stroma

ANTI-DLL4 TREATMENT OF NSCLC PDX TUMORS IN HUMANIZED MICE SHOWS IMMUNE ENGAGEMENT

Anti-DLL4 inhibition of lung tumors in huGM3 mice increases microvascular density measured by anti-CD34 immunohistochemistry

HYPOXIA-RELATED GENES ARE MODIFIED WITH ANTI-DLL4 TREATMENT IN NSCLC TUMOR AND STROMA

Hyopxia-related genes including Apln and Vasa are up-regulated in treated tumors

SUMMARY

- NSCLC-PDX tumors treated with anti-DLL4 show tumor growth inhibition.
- Anti-DLL4 treatment combined with standard-of-care carboplatin/pemetrexed in NSCLC-PDX tumors shows improved tumor growth inhibition.
- Tumor-initiating cells are decreased in NSCLC tumors treated with anti-DLL4 combined with standard-of-care.
- Notch pathway and stem cell-related genes are down-regulated in NSCLC tumors with anti-DLL4 and combination treatment.
- Previously-observed stromal genes including many vasculature-related genes are up-regulated in anti-DLL4 treated NSCLC tumors, consistent with increased vascular density measured by anti-CD34 immunohistochemistry.
- A proof-of-concept efficacy experiment in humanized mice shows NSCLC-PDX tumors can be grown in huGM3 mice and treated with anti-DLL4.
- Preliminary results from humanized huGM3 mice with anti-DLL4 treatment of NSCLC-PDX tumors show up-regulation of angiogenic tumor and human CD4+ cells as well as down-regulation of angiogenic human CD33 cells, suggesting an increased anti-tumor immune response.