ZM008 A FIRST IN CLASS MONOCLONAL ANTI LLT1 ANTIBODY DEMONSTRATED CLINICAL POTENTIAL IN MULTIPLE SOLID CANCERS

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Background Lectin-like transcript 1 (LLT1/CLEC2D) interaction with CD161 on NK cells facilitates tumor immune escape.1, 2, 3 Others and we have shown that anti LLT1 antibody disrupts LLT1-CD161 interaction to release the break on NK cells, activate NK cells, improve immune cell infiltration and enhance tumor cell cytotoxicity.4, 5, 6 Present work describes a novel anti LLT1 antibody ZM008, it’s mode of action, GLP safety and toxicity studies, in vivo and ex vivo anti-tumor effects.

Methods Cytokine release and immune cell activation were monitored using human PBMC. PC3 xenografted HuNOG-EXL mice were treated with ZM008 to monitor anti-tumor effects and tumor IHC was performed to study the TME. 4 weeks repeat dose GLP study in Cynomolgus monkey was conducted to determine safety and toxicity of ZM008 at 10-125mg/kg dosing. 3D ex vivo culture of patient biopsies from NSCLC and muscle invasive bladder cancer (MIBC) were used to determine efficacy of ZM008.

Results ZM008 induced CD69 activation and perforin/granzyme B expression on cytotoxic CD8+ T cells and CD16+ NK cells and promoted release of IFNγ and TNFα from immune cells. In HuNOG-EXL-PC3 xenograft mice, ZM008 treatment resulted in ~48% tumor growth reduction and significant tumor infiltration of CD8+ T cells, CD56+ NK cells and NKG2D+ cells was observed. Additionally, low Ki67 and high caspase 3 expression after ZM008 treatment indicates antitumor effects (figure 1). ZM008 exposure was maintained over the treatment period with no mortality or toxicity and was well tolerated in 4 weeks repeat intravenous dosing in Cynomolgus monkeys. No ZM008 related gross pathological findings, organ weight changes or histologic lesions were observed. 3D tumoroid culture from biopsies of NSCLC and MIBC patients were treated with ZM008 monotherapy and Pembrolizumab combination therapy revealed >50% reduction of tumoroids. High-content imaging of the tumoroid clearly shows disintegration of TME and infiltration of immune cells (figure 2).

Conclusions This is the first report describing anti LLT1 antibody efficacy in patient biopsy samples. Tumoroids derived from lung cancer and bladder cancer biopsies revealed significant growth reduction and immune cell infiltration with ZM008 treatment. ZM008 disrupts LLT1-CD161 pathway and transforms the TME to immune responsive “Hot” tumor by activation of immune cells, cytokine secretion, granzyme B/perforin release, and tumor cytotoxicity. GLP safety and toxicity studies in Cynomolgus monkey support future clinical application of ZM008. Overall, the data suggests ZM008 drug product is poised to initiate phase 1 trial (monotherapy and combination with Pembrolizumab) in multiple solid cancers.

REFERENCES

Ethics Approval Ethics Approval of preclinical study: This study was approved by the Institutional Animal Ethics Committee IAEC Protocol Approval No: SYNGENE/IAEC/1140/02-2020. Institutional Animal Care and Use Committee (IACUC) responsible for the Testing Facility’s compliance with applicable laws and regulations concerning the humane care and use of laboratory animals. Ethics Approval for GLP safety and toxicity study: “This study is approved by Altsasciences Preclinical Services Institutional Animal Care and Use Committee (IACUC protocol number – 162822-01)” Ethics Approval for Ex Vivo study: “This study was approved by the National Bioethics Committee in Romania, approval number 95/4 from 25.11.2019.”

Abstract 1391 Figure 1 Transformation of tumor microenvironment (TME) in HuNOG-EXL mice bearing PC3 tumors upon ZM008 treatment PC3 xenografted HuNOG-EXL mice were treated with 10 mg/kg ZM008 and vehicle control intraperitoneally. In vivo efficacy of ZM008 revealed nearly 48% tumor growth reduction in animals treated with ZM008. Animals were humanely sacrificed intermittently to observe effects of ZM008 on TME. Immunohistochemistry (IHC) of cryopreserved tumor sections was carried out to determine infiltration of human CD8+, CD56+ and NKG2D+ immune cells. In addition, the tumor sections were stained with antibodies against Ki67 and Caspase 3. Critical cytokines and chemokines were also evaluated from peripheral blood of these animals. A. Tumor section images from ZM008 treated animals suggest significant infiltration of CD8+ T cells, CD56+ NK cells and NKG2D+ immune cells (red arrows) compared with control arm. In addition, low Ki67 and high caspase 3 staining (red arrows) were observed in these tumors indicating anti-tumor effects of ZM008 treatment. B. Bar diagram showing increased infiltration of CD8+ T cells and CD56+ NK cells and NKG2D+ immune cells whereas significant reduction of Ki67 and caspase 3 on the tumor cells. C. Cartoon showing overall changes observed in the TME of animals treated with ZM008. The representation indicates transition to "Hot" immunologically responsive TME as evidenced by increased infiltration of CD8+, CD56+, NKG2D+ immune cells and decreased Treg cells; low

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Ki67 and high Caspase-3 levels in tumor cells; release of proinflammatory cytokines like IFNγ and TNFα after ZM008 treatment.

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Efficacy of ZM008 was determined as monotherapy or in combination with Pembrolizumab using Ex Vivo Clinical Trial's (EVCT) Platform. Freshly isolated patient biopsy samples from Non-Small Cell Lung Cancer (NSCLC) and muscle invasive bladder cancer (MIBC) indications were co-cultured with labeled autologous PBMCs. 3D cell culture system was used to culture the tumoroid in presence or absence of ZM008 or Pembrolizumab. Pembrolizumab was used at 20 μg/mL and ZM008 was used at 5, 20 and 50 μg/mL concentrations. Separate wells were used to test the effects of ZM008 and Pembrolizumab combinations. Quantitative image analysis of tumoroid area and tumoroid count were performed to evaluate the effects of ZM008 treatment.

A. Overall responses of ZM008 monotherapy and combination therapy with Pembrolizumab in terms of tumoroid area and tumoroid count in NSCLC and MIBC biopsy samples. The data is represented as percentage change from mean value of the control set (red line). The upper dotted line represents 20% increase of tumoroid area and count, responses above this line indicates tumor progression. The lower dotted line represents 33% decrease in tumoroid area and count, below this line could be considered as partial response to ZM008. The grey shaded area represents no significant change in tumoroid area and count, could be considered similar to stable response. Analysis of the data suggests ZM008 monotherapy and combination therapy with Pembrolizumab revealed significant reduction of tumoroid area and count. This indicates anti-tumor effects of ZM008 on patient biopsies in solid cancers.

B. Few samples were chosen for high content image analysis using confocal microscopy. Tumor cells in the tumoroid structures were indicated by actin staining (red), autologous PBMCs were stained with Cell tracker (green) and nuclei were stained with DAPI (blue). Images represent maximum projection of confocal stacks taken with 20X water immersion (WI) objective and images were processed using ImageJ. Representative image analysis showing significant reduction in tumoroid area upon treatment with ZM008 monotherapy at 50 μg/mL as well as in combination with Pembrolizumab. Reduction in size of tumoroid was monitored by disintegration of actin signal. Immune cell infiltration was represented by presence of green cells within the tumoroid structures in samples treated with ZM008 monotherapy as well as ZM008 +Pembrolizumab combination therapy.