Generation and Characterization of Antibodies Specific for IL-1RAP Antigen To Target Quiescent and Proliferating AML Leukemic Stem Cells

Ping Jiang¹, Jen Huang², Jeff Lin¹, Jennifer Lu¹, Sharmili Ambudkar¹, Roy¹, Xiaoxian Zhao¹, Eric D Hsi² and Tim Feng³

¹Cellerant Therapeutics, Inc, San Carlos, CA, ²Cleveland Clinic, Cleveland, OH

ABSTRACT

We have identified several cell surface targets preferentially expressed on primary AML blast and leukemic stem cells (LSCs) that are not expressed or expressed at low levels on normal hematopoietic stem cells. We isolated and characterized AML blast and LSCs from AML patients and immunized mice to produce a family of potent, specific, monoclonal antibodies (mAbs) against interleukin-1 receptor accessory protein (IL-1RAP), one of the AML LSC specific targets we identified.

mAb clones were screened, characterized and evaluated in vitro and in vivo for primary patient AML and IL-1RAP+ cell line binding properties. In addition, internalization after binding of several clones was investigated. These mAbs demonstrated preferential binding profiles to primary AML patients’ samples and robust anti-tumor activity in cell based assays in vitro and a tumor xenograft model in vivo. Several selected clones have a high degree of specific binding and killing of primary AML patient samples (>90%) in a complement-dependent cytotoxicity assay. Several IL-1RAP mAb clones evaluated have shown potent inhibition of tumor engraftment and growth in an orthotopic AML xenograft tumor model. In summary, the binding profiles and in vitro and in vivo efficacy against patient and AML cells indicates that targeting IL-1RAP is a promising therapeutic approach for the treatment of AML, and has the potential for effective eradication of AML LSCs and long term remission in the clinic. These results highlight the potential use of mAbs for generation of conjugates suitable for therapeutic use.

METHODS

ELISA analysis by Flow Cytometry. BMMC or PBMC were isolated from bone marrow aspirate or whole blood by Ficoll separation. Cells were resuspended in staining solution and blocked with 0.1% gelatin or mouse IgG. A total of ten random primary AML samples were scored. A CD38-APC was added followed by addition of Dye488 conjugated antibodies. Antibodies incubated in BMMC or PBMC for one on for 30 min. Cells were washed and resuspended in 200 µL of staining solution with propidium iodide. Cells were analyzed by flow cytometry. A sample was considered positive for target if the ratio of the geometric mean fluorescence intensity of the stained sample and 1% control was greater than 1.5 and more than 5% of the cells expressed the antigen compared with the control sample.

Complement-Dependent Cytotoxicity: Cells were washed and dead cells removed using Live/Dead Kit. Cells were resuspended to a final concentration of 0.8 x 10^5/mL. 50 µL of the 50% of the 2X dilution antibody was mixed and allowed to incubate for 10 minutes at room temperature prior to addition of 10µL baby mouse complement. Control was cell plus complement. The plate was incubated at 37°C for 2 hours before allowing to rest at room temperature for 10 minutes. 100µL of Cell Titer-Glo was added and allowed to shake for 5-10 minutes prior to reading on a luminescent plate reader.

Internalization assay: 2500 cells/50 uL of IL-1RAP 293 were plated in 96-well microtiter plates. Primary antibodies and Mab-ZAP were added in a volume of 50 µL. The plates were incubated 72 hours at 37°C in the presence of 5% CO2. For each plate, 100 uL of Cell Titer-Glo (Promega) was added and allowed to shake for 5-10 minutes prior to reading on a luminescent plate reader. Data was analyzed using GraphPad Prism. A 4-parameter curve is generated using concentration and percent of cells killing.

ELISA activity in orthotopic AML xenograft engraftment model. 5 x 10^6 viable EOL cells injected IV into NOD/SCID mice. mAbs dosing started 1 day prior to tumor challenge. 10µg/mg QD, 7 doses total N=6 mice/group.

RESULTS

Figure 1. Expression of IL-1RAP in normal HSC and AML.

(A) A bone marrow sample from (A) a healthy volunteer (B) an AML patient sample was stained with CD34, CD38 and IL-1RAP antibodies. Shown are histograms of the percentage of cells expressing IL-1RAP antibody (red line).

(A) Aggregated data from IL-1RAP mAb staining of AML patient samples segmented by FAB subtypes and NCCN cytogenetic risk category (B) Several anti-IL1RAP clones were screened for binding AML patient samples.

(A) Fluorescence intensity of AML cell lines stained with clone 12G6 (green line) or isotype control (blue line). (B) Representative flow cytometry dot plots and histograms showing binding of IL-1RAP clones 12G6 and 3G7 on AML PBMC.

(A) CDC activity of IL-1RAP antibodies against primary AML blasts.

(A) CDC induced by mAbs, AML PBMC were incubated with increasing concentrations of mAbs in the presence of baby rabbit complement. (Blank) plot of IL-1RAP mean fluorescence index vs CDC potency. A straight line was plotted to determine if a correlation between MFI vs % death could be identified

(A) Twenty-six mAbs were tested for internalization activity by using mAbZAP as a conjugated secondary reagent. The blue bar represents the Log Antibody (µg/mL) and purple bar represents mAb concentration at 2µg/mL and 60 µg/mL. (A) A total of five mAbs were selected that bind to various epitopes and tested for in vitro activity in bone marrow (A), spleen (B) and blood (B) of animals transplanted with an aggressively growing AML cell line EOL-1.

Figure 6. In Vivo Activity of IL-1RAP mAb-clones in the orthotopic EOL-1 AML xenograft engraftment model.

SUMMARY

We have demonstrated that IL-1RAP is a promising therapeutic target on AML LSCs. An initial family of antibodies generated against IL-1RAP have demonstrated specific binding and CDC mediated killing of AML cells from patients. Additionally, these antibodies have shown inhibition of AML tumor cell line engraftment and outgrowth in an orthotopic xenograft model. These data support further development of IL-1RAP targeting antibodies for the treatment of AML patients.

We have isolated and characterized AML blast cells and leukemic stem cells (LSCs) from primary AML patients and identified surface targets selectively expressed on AML blast cells and LSCs and not their normal hematopoietic stem cell (Fig. 1).

Candidate mAB clones were screened on 74 AML patient samples and 70 (94.6%) of the samples were bound by at least one clone (Fig. 2A). The pool of candidate clones did not favor any specific FAB subtype or any cytogenetic risk category (Fig. 2B).

C10D6 and 3G7 were further evaluated for binding to several AML cell lines (Fig. 3A) and additional AMP patient samples (Fig. 3B).

Preliminary results from complement dependent cytotoxicity experiments suggest variability of efficacy between various mAb clones. Clone C10D6 exhibited a strong dose-dependent killing of primary AML cells in addition to near complete detection of IL-1RAP surface expression on blasts (Fig. 4A). Our initial results indicate that IL-1RAP targeted killing of primary AML blasts is not correlated with IL1RAP expression levels (Fig. 4B).

Candidate clones were tested for internalization on IL-1RAP expressing 293 cells at 2 µg/mL concentration and several were shown to be efficiently internalized (Fig. 5A). Additionally, several clones showed promising in vivo cell killing using a piggyback against conjugated goat anti-mouse secondary reagent. For these three clones tested, the internalization and killing was dose dependent (Fig. 5B).

A total of five clones were selected that bound to various epitopes on IL-1RAP and tested for in vivo inhibition of tumor engraftment activity. All antibody clones showed in vivo activity with more than 90% inhibition of tumor engraftment in bone marrow, spleen and liver samples of animals injected with an aggressively growing AML cell line (Fig. 6).