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Targeting IL-15 in large granular lymphocyte leukemia

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Abstract

IL-15 is a cytokine that stimulates the proliferation of NK and T cells. Previous studies have shown that IL-15 is critical to the induction of T-cell large granular lymphocyte (T-LGL) leukemia. A Phase I trial of a humanized antibody (Hu-Mik β 1) to the IL2/IL15R β receptor, expressed on T-LGL, is explored in this trial to evaluate the safety, pharmacokinetics, specificity and clinical efficacy of Hu-Mik β 1. The study demonstrated no toxicity and favorable saturation of IL2/IL15R β receptor, but no clinical efficacy in this Phase I study.

Keywords

IL-15; large granular lymphocyte leukemia; Phase I clinical trial

Large granular lymphocyte (LGL) leukemia is a class of rare lymphoproliferative disorders. The T-cell form (T-LGL) is CD3⁺ and is classified by the WHO as a mature peripheral T-cell neoplasm [1]. T-LGL generally runs an indolent clinical course and frequently presents with neutropenia, anemia and rheumatoid arthritis. A computational systems biology approach was previously used to identify IL-15 and PDGF as master regulators of leukemic LGL survival, demonstrating that dysregulation of these cytokines could explain all known molecular features of LGL leukemia [2]. Furthermore, dysfunctional activation-induced cell death due to chronic viral infection or antigen stimulation is thought to be critical to disease pathogenesis. Multiple survival pathways are known to be dysregulated in LGL leukemia including JAK/STAT [3], sphingolipid [4], MAPK [5] and PI3K/AKT [6] signaling. More recently, STAT3 mutations have been shown to occur in approximately 40% of patients with T-LGL leukemia [3].

Treatment of LGL leukemia patients with immunosuppressive agents such as low-dose methotrexate, cyclophosphamide or cyclosporine has led to an overall response rate of approximately 50% in retrospective studies. Our recently conducted Eastern Cooperative Oncology Group trial was the first prospective clinical trial in LGL leukemia, demonstrating a 39% response rate to methotrexate as initial therapy [7]. Treatment with an anti-CD52 antibody (alemtuzumab) showed a response rate in four out of eight patients with refractory LGL leukemia [8]. Clearly, there is an urgent need for development of better therapeutics. Other potential targets include FasL, PDGFR, STAT3, NF- κ B, the sphingolipid rheostat,

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MAPK and PI3K/AKT signaling [1]. This study evaluates therapy targeting the IL-15 receptor [9].

IL-15 is a member of the IL-2 family that regulates T- and NK-cell activation and proliferation. IL-15 signals through a heterotrimeric receptor to activate downstream signals including JAK/STAT and MAPK signaling [10]. Monocytes and dendritic cells express the IL-15-specific subunit (IL15R α) and secrete IL-15 locally. IL15R α presents IL-15 to the other two receptor subunits, IL-2/IL-15R β (specific to IL-2 and IL-15) and γ -c common subunit (shared among multiple interleukins), which are expressed on NK and cytotoxic T cells. The presentation of IL-15 from one receptor subunit on one cell type to the two other receptor subunits is known as *trans* presentation of IL15. Interestingly, it has recently been identified that in some patients with T-LGL leukemia, IL-15R α is expressed in the T-LGL cells, leading to all three subunits being expressed in *cis* [11]. Dysregulated IL-15 has been reported in patients with autoimmune diseases (e.g., rheumatoid arthritis, multiple sclerosis and Celiac's disease), as well as HTLV-1 infection [12].

Summary of preclinical results

Previously, the authors conducted a Phase I clinical trial with a murine antibody to IL-2/IL-15R β ; however, no efficacy was observed, which the authors believed was due to its murine origin [13]. To address the proposed issues from the previous trial, the authors developed a humanized form of Mik β 1 [14].

The preclinical trial addressed antibody specificity, preclinical toxicology and *in vitro* functional analysis. Hu-Mik β 1 was screened for binding affinity. Out of over 30 normal human tissues screened, binding was shown to occur only against skeletal muscle specimens, which was likely due to nonspecific antibody binding. Hu-Mik β 1 did react to lymphoid cell lines but not cell lines of myeloid origin. These studies demonstrated that Hu-Mik β 1 is specific to monocyte and lymphoid lineages [9].

Preclinical toxicology was assessed in cynomolgus monkeys with varying doses and treatment periods. No clinical or laboratory abnormalities were observed in terms of routine hematology, blood chemistry and neutrophil counts. Two animals demonstrated multifocal myocardial degeneration on histology [9]. These changes did not appear to be related to the treatment regimen as similar abnormalities have been reported to occur spontaneously in these animals [15].

32D β cells (expressing only IL-2/IL-15R β) were treated with either IL-2 or IL-15, with or without Hu-Mik β 1 treatment at varying doses. Hu-Mik β 1 blocked IL-2- and IL-15-dependent cell proliferation in a dose-dependent manner. Kit225 cells (IL-2/IL-15R β and the common γ -c receptor) were next treated with IL-2 and IL-15 in the presence of Hu-Mik β 1. Cell proliferation due to IL-15 was inhibited; however, IL-2-driven cell proliferation was not inhibited. This was due to the inability of Hu-Mik β 1 to block heterotrimeric signaling when the IL-2 or IL-15 heterotrimeric receptors are expressed in *cis*. Anti-Tac, an antibody against IL-2R α was able to block IL-2-driven proliferation of Kit 225 cells; however, it was unable to block IL-15-driven proliferation. Finally, a cell line expressing the complete IL-2 and IL-15 receptors in *cis* (Kit225 7.10) was treated with IL-15 and then Hu-Mik β 1, Anti-Tac (to IL-2), or an antibody against IL-15 (Anti-IL-15). This cell line was resistant to Hu-Mik β 1 and Anti-Tac but was sensitive to Anti-IL-15 [9]. To summarize, Hu-Mik β 1 was able to block *trans* presentation of IL-15 or IL-2 but was unable to suppress *cis* presentation of IL-2 or IL-15.

Summary of clinical trial results

Nine T-LGL leukemia patients were included in this trial. The diagnosis of T-LGL leukemia was established by histology and flow cytometry (for C3⁺CD8⁺CD4⁺CD57⁺ T-LGL cell count >1000/ μ l) and required documentation of monoclonality by T-cell receptor (*TCR*) gene rearrangement. Other eligibility requirements included demonstration that over 50% of patient T cells express IL-2/IL-15R β and significant cytopenia. The authors noted that circulating mononuclear cells of patients had a high expression of IL-2/IL-15R β although they do not provide a quantitative determination.

This Phase I clinical trial was administered as an open-label study, meaning both researchers and participants knew which treatment was being administered. Patients were given a single dose of the drug intravenously at 0.5, 1.0 or 1.5 mg/kg over 90 min, with a subsequent 42-day observation period. The primary goals of this trial were to determine the safety of Hu-Mik β 1 in patients with T-LGL leukemia, to determine the dose required to saturate IL-2/IL-15R β receptor for 3 weeks and to determine pharmacokinetics and immunogenicity.

No acute toxicities due to Hu-Mik β 1 were identified. A single patient had an elevated creatine kinase-MB after intravenous administration; however, this was determined to also be elevated preadministration. A second patient died during the trial after intensive chemotherapy for concomitant acute myeloid leukemia, also thought to be independent of Hu-Mik β 1 treatment. Furthermore, only a single patient developed an immune response due to the drug administration. This patient had previously received the murine formulation of Mik β 1.

The proposed mechanism of action of Hu-Mik β 1 is binding and saturation of the IL-2/IL-15R β , blocking *trans* presentation of the IL-15 cytokine, which has been a well-established driver of T-LGL leukemia [2,16]. In order to determine receptor saturation at the various doses given, flow cytometry was completed on patients treated with Hu-Mik β 1 using a fluorochrome-labeled Hu-Mik β 1 antibody. The 0.5-mg/kg dose was insufficient to saturate the IL-2/IL-15R α receptor. However, at the 1.5-mg/kg dose, the IL-2/IL-15R β receptor was saturated over 99% for 14 days.

Patients treated with Hu-Mik β 1 did not show a clinical response, although transient increases in neutrophil counts (three patients) and platelet count (one patient) were observed. The authors further demonstrate lack of IL-2 and IL-15 production by leukemic LGL. Waldmann's group previously demonstrated that some T-LGL cases express the IL-15R α receptor in *cis*, which makes Hu-Mik β 1 ineffective [11]; however, this was not the case as cells from each patient in this study underwent proliferation when stimulated with IL-15 *ex vivo*, which was inhibited by the treatment with Hu-Mik β 1.

Discussion & significance

Waldmann, a pioneer in cytokine biology, is the codiscoverer of IL-15. Here, he reports the first-in-human Phase I trial of Hu-Mik β 1, an antibody that targets the shared IL-2 and IL-15 receptor subunit, IL-2/IL-15R β [9]. In the present study, Hu-Mik β 1 is shown to specifically target and block the *trans* presentation of IL-15 in multiple cell lines and T-LGL patient cells coexpressing the IL-2/IL-15R β and γ -c receptor subunits. However, Hu-Mik β 1 was ineffective when the α -receptor subunit for IL-2 or IL-15 was coexpressed by the leukemic cell (producing the entire heterotrimeric receptor in *cis*). Because this is a Phase I trial, the most important outcome was the toxicity profile, of which there was none. Furthermore, there were no apparent immune reactions with a single dose of 1.5 mg/kg in T-LGL patients. However, there appeared to be little efficacy in altering T-LGL disease course. This may be due to the short duration of receptor inhibition by Hu-Mik β 1.

A previous systems biology study using network analysis of a signaling network showed that IL-15 and PDGF signals can recapitulate the dysregulations seen in T-LGL leukemia, including apoptosis inhibition [2]. The present study only targets IL-15; thus, only a subset of the dysregulations may be addressed by this treatment. One possibility is that PDGF can drive LGL leukemia, regardless of IL-15 inhibition, once disease onset has occurred (although both cytokines are required during initial induction of T-LGL leukemia). A plausible solution could be to target both IL-15 and PDGF for the treatment of T-LGL leukemia. It is also possible that dysregulations other than IL-15 and PDGF could 'mimic' the leukemic state driven by these cytokines. Furthermore, chronic stimulation with IL-15 early in the disease course could allow onset of genomic instability [16] and subsequent acquisition of mutations that would make T-LGL cells become IL-15 independent. STAT3 was predicted to be a key hub in the leukemic LGL survival network [2]. Recently, we observed activating STAT3 mutations in 40% of patients with T-LGL leukemia [3]. Persistent upregulation of downstream signaling components activated by STAT3 mutation would bypass the necessity of upstream regulators such as IL-15.

Future perspective

It seems possible that Hu-Mik β 1 treatment could be modified to be more efficacious by altering drug schedules designed to keep IL-2/IL-15R β receptors saturated for a longer period of time. This will likely be the goal of future Phase II clinical trials. Additionally, it might be advantageous to look at apoptosis induction and downstream signaling as correlates in future studies. Because LGL leukemia has a chronic, indolent course, patient cells may not be dividing all that rapidly; thus, the effect of blocking cell proliferation may be hard to discern and a less important disease hallmark to target. Induction of apoptosis might be a better predictor of treatment outcome in such an indolent disease. Analysis of downstream signaling would also be important to determine whether drug treatment is in fact shutting down canonical IL-15 pathways such as JAK/STAT and MAPK signaling in these patients. Furthermore, targeting IL-15 in combination with PDGF might be an effective approach to drug treatment, since both cytokines are required for induction of the LGL disease state. Finally, since STAT3 is mutated in both chronic NK and T-LGL leukemia, targeting STAT3 could be an attractive treatment option in LGL leukemia.

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Key issues

- Hu-Mik β 1 is a humanized antibody to the IL-2/IL-15R β receptor and was shown to block IL-2- and IL-15-driven cell proliferation in preclinical models and patient leukemic cells.
- Hu-Mik β 1 was evaluated in a Phase I clinical trial in patients with T-cell large granular lymphocyte leukemia. Hu-Mik β 1 was shown to be safe in Phase I trials; however, no therapeutic efficacy was demonstrated in patients with T-cell large granular lymphocytic leukemia in this study.
- Future trials with Hu-Mik β 1 treatment could be modified to be potentially more efficacious by altering drug schedules designed to keep IL-2/IL-15R β receptors saturated for a longer period of time.