W118 The PK/PD profile and observed activity in non-human primates of HuABC2 (anti-CD122), a novel humanized antibody to the IL-15 receptor and the high and low affinity receptors for IL-2, is consistent with its potential as a safe and effective immunosuppressant

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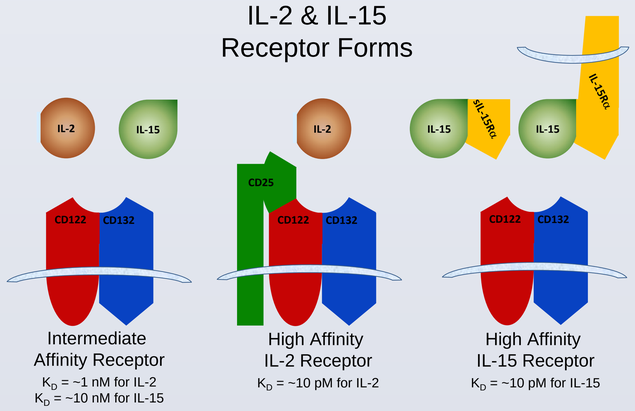
Abstract

Inhibition of IL-2 using anti-CD25 is a part of the current therapeutic regimen for prevention of organ graft rejection that also includes mycophenolic acid, corticosteroids, and a calcineurin and/or mTOR inhibitor. Anti-CD25, however, only inhibits IL-2 signaling through the high affinity receptor. Targeting CD122, which would inhibit IL-2 signaling by both the high and intermediate affinity receptors, as well as prevent IL-15 signaling, is an alternative that could provide more potent immunosuppressive potential and allow for a decrease or elimination of components of the current regimen that possess inherent toxicity. To develop a therapeutic antibody targeting CD122, a large panel of monoclonal antibodies was generated using a variety of immunogens and immunization strategies. This panel was extensively characterized for high affinity binding to human CD122, and inhibition of both IL-2 and IL-15 mediated proliferation of transfectants expressing the intermediate and high affinity IL-2 and IL-15 receptors. The most potent murine antibody was successfully humanized to generate HuABC2. A single dose 28 day PK/PD study of HuABC2 at 1 mg/kg and 10 mg/kg was carried out in cynomolgus monkeys (three per dose). HuABC2 was well tolerated and exhibited a 3-7 day serum half-life. There was no immediate cell depletion after HuABC2 infusion; however a decrease in the percentage of $CD8^+$ and $CD16^+$ cells that were $CD122^+$ occurred over time. CD122 occupancy by HuABC2 was rapid and persistent, with high occupancy at both doses. These results are consistent with the potential of HuABC2 to be a new, effective and safe immunosuppressant.

Background

CD122 is a 525 amino acid-long membrane protein expressed on T cells, NK cells, monocytes and a subset of B cells[1]. The CD122 molecule is an integral part of the receptor for interleukin 2 (IL-2) and interleukin 15 (IL-15)[2], two type I cytokines. CD122, also referred to as the β chain of the IL-2 and IL-15 receptors, combines with other receptor subcomponents to generate receptors that differ in cytokine specificity and affinity. CD122 in combination with CD132 (also referred to as the γ_{common} or γ_c chain) forms a receptor that exhibits an intermediate level of affinity for both IL-2 (K_D = ~1 nM) and IL-15 (K_D = ~10 nM). CD122 associated with both CD25 (IL-2 receptor α chain) and CD132 results in a receptor with high affinity (K_D = ~10 pM) specific for IL-2, while CD122 in combination with IL-15 receptor α chain (preliminary designation: CD215) and CD132 generates a high affinity receptor (K_D = ~10 pM) for IL-15[3].



IL-2 and IL-15 share the capacity to stimulate the proliferation of T lymphocytes, but each possesses unique activities in the maintenance of the immune system. IL-2 also plays a role to limit T cell reactivity by priming activated T cells for apoptosis, while IL-15 is required for the development of NK cells and the development and maintenance of $CD8^+$ memory T cells[2]. These two cytokines also exhibit a fundamental difference in the means by which they interact with their respective receptor components. IL-2 signaling occurs when the soluble cytokine secreted by the same or a distinct cell interacts with either the intermediate or high affinity IL-2 receptor on the surface of a cell (cis-presentation). In contrast, IL-15 signaling is carried out via presentation of IL-15 bound to IL-15Rα on the surface of one cell to another cell expressing the CD122 and CD132 molecules. This unique mechanism is referred to as trans-presentation. The affinity of isolated IL-15Rα for IL-15 is high (K_D = ~10 pM) and it is believed that IL-15 and IL-15Rα associate in the endoplasmic reticulum and are transported to the cell surface as a complex for trans-presentation to $CD122^+/CD132^+$ cells[3-5]. The vast majority of IL-15 signaling is mediated via trans-presentation, and little or no signaling occurs by cis-presentation of soluble IL-15 to IL-15 receptors[5]. In fact, evidence exists to support the hypothesis that the soluble form of IL-15 *in vivo* is not the cytokine alone, but rather IL-15 complexed to a fragment of the IL-15Rα[6]. Recently, evidence of a trans-presentation mechanism for IL-2 has been presented[7], but the biological relevance is as yet unclear.

Materials and Methods

Reagents and Cell Lines. IL-2 and IL-15 were purchased from BioVision. To approximate trans-presentation of IL-15, IL-15 was expressed linked to a fragment of the IL-15Rα chain. Specifically, the 97 amino terminal residues of IL-15Rα were joined at the carboxy terminus to the amino terminus of the IL-15 sequence by a linker consisting of the amino acid sequence SGGSGGGGSGGGSGGGGSLQ. This hybrid molecule, IL-15/IL-15$R\alpha_{fragment}$, behaved as would IL-15 bound to IL-15Rα on a presenting cell. TF-1, a human erythroleukemic cell line, was obtained from ATCC. TF-1, which endogenously expresses CD132 (γ_c chain), was transfected with CD122 to generate TF-1β. Thus, TF-1β expresses the intermediate affinity form of the IL-2 and IL-15 receptor, and is responsive to both IL-2 and IL-15, as well as to IL-15/IL-15Rαfragment (trans-presented IL-15). CD25 was introduced into TF-1β to produce TF-1αβ, which expresses the high affinity form of the IL-2 receptor and is responsive to limiting concentrations of IL-2. Human PBMC were activated by incubation with plastic bound anti-CD3 for three days and washed prior to use in proliferation assays.

HuABC2 generation and characterization. A panel of over 160 murine monoclonal antibodies against human CD122 was generated using a variety of immunogens and immunization protocols. Hybridoma supernatants were screened by ELISA to detect binding to CD122. Antibody was purified from hybridomas producing positive supernatants and thoroughly characterized for the ability to inhibit IL-2 and IL-15 mediated proliferation using TF-1β and TF-1αβ. These lines provided the capacity to analyze antibody inhibition of the stimulatory capacity of both IL-2 and IL-15 on all receptor configurations. ABC2 was the antibody in the panel that displayed the most potent ability to inhibit IL-2 mediated proliferation of TF-1β and TF-1αβ, and IL-15 mediated proliferation of TF-1β. ABC2 was humanized using methods previously described[8] to generate HuABC2. Analysis of binding and functional activity confirmed that HuABC2 retained the characteristic of the original ABC2 murine antibody.

Materials and Methods *continued*

Primate Pharmacokinetic/Pharmacodynamic/Toxicology Study. Two groups of three normal cynomolgus monkeys (*Macaca fascicularis*), 4.0-5.5 kg body weight, aged 3-6 years old, were given a single intravenous HuABC2 infusion of either 1 or 10 mg/kg. All animals were weighed at the study initiation, then once a week until the end of the study. For pharmacokinetic evaluation, 1.0 ml blood was harvested on Day -2, 0 (1 hour post-administration), 1, 3, 7, 14, 21, 28 and allowed to clot for no more than two hours. Serum was harvested, centrifuged and stored at -80 C, until all samples had been collected. Serum HuABC2 concentration was determined using an anti-idiotype based ELISA. On Day -2, 3, 14 and 28, a 0.5 ml peripheral blood was collected for hematological examinations that included red blood cell and white blood cell counts, as well as differential, hemoglobin, hematocrit, and platelets counts. On Day -2, 14 and 28, a 0.5 ml blood sample was collected into tubes without anticoagulant for biochemistry tests, that included serum creatinine, blood urea nitrogen, total protein, glucose, amylase, cholesterol, triglyceride, lactate dehydrogenase, albumin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, total bilirubin, and creatinine kinase. On Day -9, -2, 3, 14 and 28, peripheral blood was collected into EDTA tubes for flow cytometry. Flow cytometry was used to monitor CD122 expression on $CD3^+$, $CD8^+$ and $CD16^+$ cells prior to, and following HuABC2 administration. CD122 was detected via staining with the anti-human CD122 antibodies Mik-β2 or Mik-β3. Using these two antibodies, which differ in their capacity to be inhibited by HuABC2, also allowed for the evaluation of the occupancy of HuABC2 on $CD3^+$, $CD8^+$ and $CD16^+$ cells. All of the antibodies used in the analysis were of the murine IgG1 isotype, except Mik-β2, which is a murine IgG2a. All antibodies were purchased from BD Biosciences.

Results

HuABC2 has potent inhibitory activity against IL-2 and IL-15 mediated *in vitro* proliferation

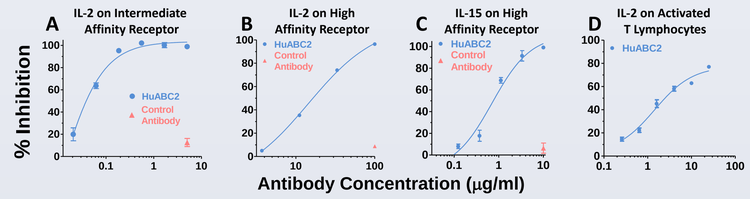
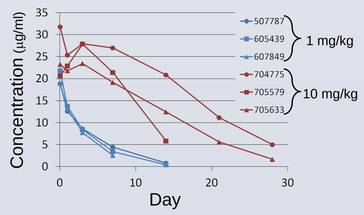


Figure 1. Varying concentrations of HuABC2 were tested for the capacity to inhibit proliferation of TF-1 transfectants to IL-2 or IL-15. *Panel A*: The inhibition of IL-2 mediated proliferation of the TF-1β cell line, which expresses the intermediate affinity IL-2/IL-15 receptor (CD122 and CD132). The concentration of IL-2 used was 200 ng/ml. The $IC_{50\%}$ of HuABC2 in this assay is 40 ng/ml. *Panel B*: The inhibition of IL-2 mediated proliferation of TF-1αβ, which expresses the high affinity IL-2 receptor (CD25, CD122 and CD132). The concentration of IL-2 used in the assay was 10 ng/ml. The $IC_{50\%}$ of HuABC2 in this assay is 14 μg/ml. *Panel C*: The inhibition of proliferation mediated by trans-presented IL-15 on TF-1β. This corresponds to inhibition of IL-15 on the high affinity IL-15 receptor. The concentration of IL-15/IL-15$R\alpha_{fragment}$ used was 10 ng/ml. The $IC_{50\%}$ of HuABC2 in this assay is 140 ng/ml. *Panel D*: The inhibition of IL-2 mediated proliferation of anti-CD3 activated human PBMC. The cell population is heterogeneous in regards to receptor type expression, but most likely the high affinity IL-2 receptor predominates. The concentration of IL-2 used in the assay was 10 ng/ml. The $IC_{50\%}$ of HuABC2 in this assay is ~2.1 μg/ml.

Pharmacokinetics of HuABC2 following a single dose at two dose levels



Dose (mg/kg)	Animal #	C_{max} μg/mL	T_{max} days	$T_{1/2}$ days	AUC_{last} μg-d/mL	AUC_{INF} μg-d/mL
1	507787	18.83	0.04	3.22	81.12	84.9
	605439	21.8	0.04	2.5	76.54	77.97
	607849	18.92	0.04	2.52	57.01	66.2
	Mean	**19.85**	**0.04**	**2.75**	**71.56**	**76.36**
	SD	1.69	0	0.41	12.8	9.46
10	704775	31.73	0.04	6.83	526.55	576
	705579	27.86	3	4.7	265.65	305.02
	705633	23.4	3	4.85	350.85	362.59
	Mean	**27.66**	**2.01**	**5.46**	**381.02**	**414.54**
	SD	4.17	1.71	1.19	133.04	142.77

Figure 2. The serum concentration of HuABC2 following a single intravenous administration at either 1 or 10 mg/kg in cynomolgus monkeys. Serum samples were prepared on Day -2, 0 (1 hour post-administration), 1, 3, 7, 14, 21, 28, stored and analyzed together for the concentration of HuABC2 by an anti-idiotype-based ELISA. PK parameters were calculated using non-compartmental methods, linear trapezoidal method for AUC, terminal slope estimated by linear regression through 3 or more points in terminal phase, AUC extrapolated to infinity (AUC_{inf}).

Serum biochemistry plots following a single exposure to HuABC2 at two dose levels

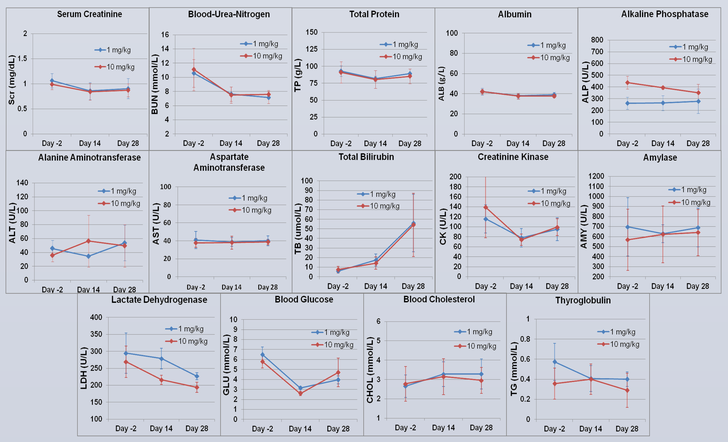


Figure 3. Plots of various serum biochemistry parameters two days prior to, as well as fourteen and twenty-eight days following HuABC2 administration. All parameters remained within normal ranges following treatment, with the exception of bilirubin, for which 2/3 animals in the 1 mg/kg dose and 2/3 animals in the 10 mg/kg dose groups exhibited a level outside of the normal range.

Results *continued*

$CD8^+$ and $CD16^+$ cells, but not $CD3^+$ cells, decrease following HuABC2 administration

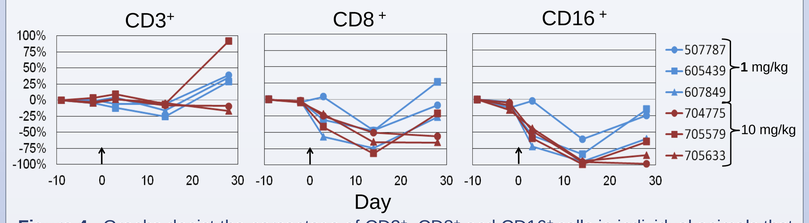


Figure 4. Graphs depict the percentage of $CD3^+$, $CD8^+$ and $CD16^+$ cells in individual animals that were $CD122^+$ on days minus nine, minus two, three, fourteen and twenty-eight days following HuABC2 administration. There was no significant change in the number of $CD3^+$ cells that were $CD122^+$ during the course of the study. However the average number of $CD8^+$ cells that were $CD122^+$ decreased from ~21% pre-infusion to ~10% on Day 14 in the 1 mg/kg dose group, and from ~27% to ~10% in the 10 mg/kg dose group. The effect on the number of $CD16^+$ cells that were $CD122^+$ was more pronounced, decreasing from ~11% pre-infusion to ~2% on Day 14 in the 1 mg/kg dose group, and from ~12% to <1% in the 10 mg/kg dose group. In all cases, the 28 day time point indicated a trend toward recovery, with the recovery being dose dependent in nature (larger recovery in lower dose).

Occupancy of CD122 by HuABC2 following a single exposure at two dose levels

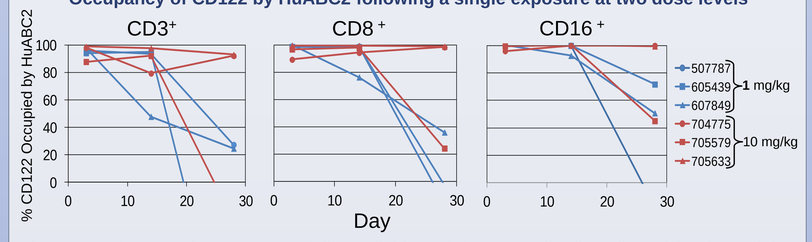


Figure 5. The use of two commercially available anti-CD122 antibodies allowed for determining the occupancy of CD122 by HuABC2 following antibody administration. Mik-β2 and Mik-β3 are anti-CD122 antibodies that differ in the ability to be blocked by HuABC2 binding; Mik-β2 is blocked, Mik-β3 is not. The graphs depict the percentage of CD122 occupied by HuABC2 on $CD3^+$, $CD8^+$ and $CD16^+$ cells in individual animals at three, fourteen and twenty-eight days following HuABC2 administration. High occupancy of CD122 by HuABC2 was established immediately after infusion at either dose on $CD3^+$, $CD8^+$ and $CD16^+$ cells, and reached nearly complete saturation. The high HuABC2 occupancy rate was maintained on Day 14 post-infusion, particularly on $CD16^+$ cells, with no clear difference between dose groups. On Day 28 post-infusion, HuABC2 occupancy of 1 mg/kg dose group had largely disappeared on $CD3^+$ and $CD8^+$ cells (-31.23% and 5.95% respectively), but higher occupancy of CD122 was detected on $CD16^+$ cells (34.99%). In the 10/mg/kg dose group, HuABC2 occupancy was 52.31%, 73.98%, and 81.45% for $CD3^+$, $CD8^+$ and $CD16^+$ cells respectively.

Discussion

Although antibodies against CD122 were first generated over twenty years ago, they were primarily selected for binding to CD122, and then subsequently characterized for any functional activity[9-11]. These antibodies were generated well before both the tri-molecular nature of the IL-2 receptor and the existence of IL-15 was established; thus functional characterization at the time was limited to inhibition of activated T cell proliferation. HuABC2 is the humanized version of a murine antibody selected from a panel of over 160 anti-CD122 antibodies. The criteria for selection, in addition to binding to CD122 with high affinity and specificity, were potent inhibitory activity against IL-2 and IL-15 on all functional forms of their respective receptors. HuABC2 maintains these activities of the original murine antibody, making it an excellent therapeutic agent for an anti-CD122 strategy. The therapeutic potential of HuABC2 for prevention of organ transplant rejection seems high. In addition to the expanded activity against IL-2 as compared to anti-CD25, the inhibition of IL-15 activity should also be beneficial, as inhibition of IL-15 is efficacious in an animal model of transplant rejection[12]. In addition, HuMik-β1, a humanized version of one of the first anti-CD122 antibodies, possesses IL-2 and IL-15 inhibitory activity significantly inferior to that of HuABC2, but was capable of prolonging cardiac allograft survival in non-human primates[13]. These results are consistent with HuABC2 having potential as a novel and effective immunosuppressant.

Summary of HuABC2 Characteristics

- A humanized IgG1/kappa monoclonal antibody against human CD122
- Exhibits a K_D of ~1 nM for human CD122
- Targets both IL-2 and IL-15 providing a unique, first-in-class therapeutic mechanism of action
- Exhibits potent inhibitory activity against known functions of IL-2 and IL-15
- Possesses reactivity to primate (cynomolgus) CD122
- Was well-tolerated in a non-human primate PK/PD study (single dose at two dose levels)
- Exhibited a 3-7 day half life in non-human primates
- Exhibited a clear pharmacodynamic effect after a single dose in non-human primates
- Is covered by pending patent applications (US: 20110250213; International: WO/2011/127324)

References

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