Clinical and Preclinical Activity of SL-401, a Targeted Therapy Directed to the Interleukin-3 Receptor on Cancer Stem Cells and Tumor Bulk, Against Blastic Plasmacytoid Dendritic Cell Neoplasm (BPDCN)

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INTRODUCTION

Blastic plasmacytoid dendritic cell neoplasm—BPDCN is a malignancy of plasmacytoid dendritic cells characterized by frequent skin, lymph nodes, bone marrow and spleen involvement first described in 1994 (Chaperot, Blood 97, 3210, 2001). With the discovery of a distinctive phenotype—CD4+/CD56+/CD123+, the frequency of diagnosis has increased in the last decade such that approximately 1% of leukemias plus 0.5% of lymphomas have this diagnosis (Pagano, Haematologica 98, 239, 2013). Thus, while rare, the incidence of this disease exceeds several thousand worldwide each year. There is no standard treatment for BPDCN; treatment often consists of intensive combination chemotherapy used to treat either AML or aggressive lymphoma, which is often followed by allogeneic stem cell transplantation when feasible (Roos-Weil, Blood 121, 440, 2013). Nevertheless, most patients relapse with chemoresistant disease. In the largest retrospective series to date, consisting of 90 patients, Julia et al. reported a mean overall survival of 12 months (Julia et al., Brit J Derm 2013 May 4; epub ahead of print). Elderly BPDCN patients not receiving aggressive combination chemotherapy or transplants have a more dismal outcome.

Because of the extremely high levels of CD123 – the interleukin 3 receptor α subunit on BPDCN blasts – we hypothesized that a fusion protein targeting the high affinity interleukin 3 receptor would be a novel therapy for this disease. In 2000, we fused DNA encoding diphtheria toxin (DT) amino acid residues 1-388, a Met-His linker, and human interleukin 3 (IL3), transformed *E. coli*, induced expression with IPTG, and purified $DT_{388}IL3$ designated SL-401 from inclusion bodies by denaturation, refolding, anion exchange chromatography and size exclusion chromatography (Frankel, Protein Eng 13, 575, 2000; Figure 1).

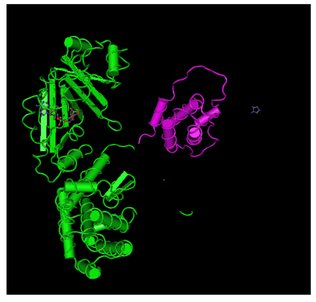


Figure 1. SL-401 putative 3-dimensional structure. Green (top) is DT catalytic domain; Green (bottom) is DT translocation domain; Magenta is huIL3. Adapted from Bennet, Protein Sci 3, 1444, 1994 and Feng, JMB 59, 524, 1996.

SL-401 cytotoxicity to AML blasts was proportional to the density of IL3Rα, IL3Rβ, and high affinity IL3R (Alexander, Bioconj Chem 11, 564, 2000; Frankel, Leukemia 14, 576, 2000; Alexander, Leuk Res 25, 875, 2001; Testa, Blood 106, 2527, 2005; Yalcintepe, Blood 108, 3530, 2006; Hogge, Clin Cancer Res 12, 1284, 2006; Figure 2).

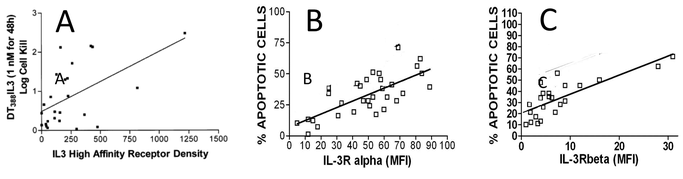
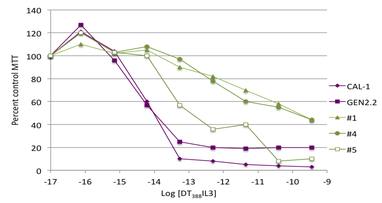


Figure 2. A. Patient blast methocult colony SL-401 inhibition and ^{125}I-SC-50341 binding . B. Patient blasts flow with anti-CD123 (IL3-Ralpha) and separately SL-401 incubation 24h and annexin V binding. C. Same as B except anti-CDw131 (IL-3Rbeta) flow. MFI, mean fluorescence intensity.

We then incubated BPDCN cell lines and fresh blasts with SL-401 for 24h and assayed cytotoxicity by MTT assay (Angelot-Delettre, Blood 118 Suppl, 2588, 2011; Figure 3). All BPDCN samples showed sensitivity to drug.

Figure 3. SL-401 was incubated 24 h with BPDCN cell lines (GEN2.2 or CAL-1) or patient blasts (#1, #4, and #5) and assayed by MTT.



TRIAL DESIGN

Single site, single dose level, single cycle expansion cohort involving patients with BPDCN, which was part of a larger 86 patient phase 1-2 study of SL-401 study (Frankel et al, J Clin Oncol 31 Suppl, 7029, 2013; presentation at ASCO 2013; Abstract 7029). Eligibility criteria include: age ≥ 18, histologically confirmed BPDCN, good organ function (bilirubin ≤1.5mg/dL, SGOT/SGPT ≤2.5xULN, albumin ≥3g/dL, creatinine ≤1.3mg/dL, cardiac ejection fraaction ≥50%), ECOG performance status ≤2. Exclusion criteria include: no uncontrolled infections, DIC, pregnancy, concurrent relevant medical illnesses, CNS disease, or a recent MI or CHF. Patients have received five daily doses of SL-401 12.5µg/kg (5 patients) or 9.4 µg/kg (1 patient), which is equivalent to one cycle. All patents have received only a single 5-day cycle. SL-401 was administered IV over 15 min after premedication with solumedrol, acetaminophen, diphenhydramine and ranitidine. Interval histories, physical exams, CBCs, chemistries have been performed daily for one week then weekly thereafter for 4 weeks. Toxicity was graded according to CTCAE v4.0. Response was assessed with pretreatment and posttreatment CBC/Diff, bone marrow exams, skin exams/biopsies and PET/CT scans. Pharmacologic (SL-401 serum levels), immunologic (anti-SL-401 antibody levels), and translational (marrow blast CD123 density) studies have been performed.

RESULTS

Seven patients with BPDCN were screened, and six patients treated (Table 1). Median age was 59 years (range 35-72). There was one female and 5 males. Disease was de novo in 1 patient, first relapse in 1 patient, second relapse in 1 patient and post-transplant in 3 patients. Drug-related toxicities were transient and mild to moderate. There were no grade 3 toxicities. The grade 2 toxicities consisted of fever, chills, hypotension, hypoxemia, transaminasemia and hypoalbuminemia (Figure 4). Cmax and half-life ranged from 0 to 22ng/mL and 30-50 min; anti-SL-401 pretreatment antibody levels ranged from 0 to 8 µg/mL (Figure 5; Table 2). Among 6 patients evaluable for response, there were 5 responders, including 3 CRs, one CR of which was on-going (lasting 9+ mo and ongoing), one CR lasted 5 mo, one CR lasted 1 mo, one PR lasted 1 mo, and one PR is currently ongoing at 0.5+ mo. All evaluable patients demonstrated clearance of marrow and peripheral blasts, adenopathy, splenomegaly, and partial or complete clearance of skin lesions (Table 2). Patient accrual is continuing.

Table 1. Relevant Clinical Information

Subject No.	Age/ Gender	Previous Treatment	Sites of Disease	No. of Doses Received of 5 Daily Doses (1 cycle)
1	35/F	Two intensive combination chemotherapy regimens	Bone Marrow	5/5
2	40/M	Cytarabine/Daunorubicin/Etoposide, BMT, DLI	Bone Marrow, Nodes	5/5
3	72/M	Cytarabine/Idarubicin, Gemcitabine, BMTx2	Skin, Bone Marrow	3/5
4	65/M	Etoposide/Doxorubicin/Vincristine/Prednisone/Cyclophosphamide, Fludarabine, BMT	Skin, Bone Marrow	5/5
5	70/M	Decitabine	Skin,Bone Marrow	5/5
6	70/M	0	skin	5/5

Figure 4. Grade 2 Toxicities

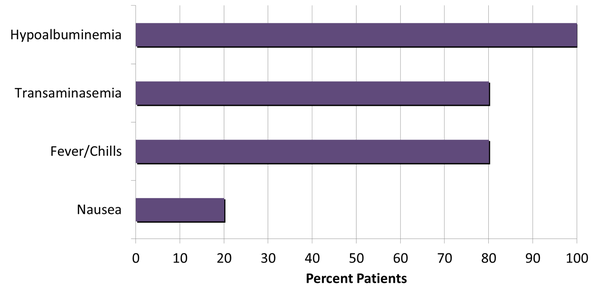
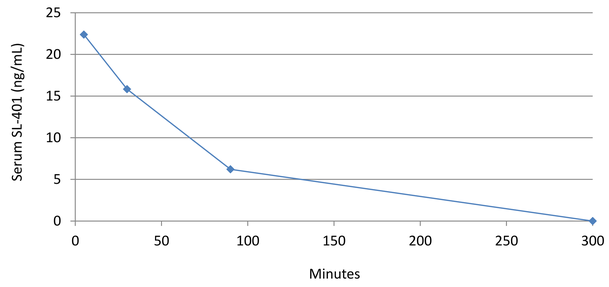


Table 2. Patient PK/Immune Response and Clinical Response

Subject No	Cmax days 1/5 (ng/mL)	Half-lives days1/5 (min)	Antibody pre/day 15-30 (µg/mL)*	Objective Response*	Response Duration (mo)
1	ND	ND	ND	ND	ND
2	0/7	-/50	6/34	CR	5
3	0/-	-/-	8/4590	CR	9+ (ongoing)
4	22/2	40/10	0.5/35	CR	1
5	8/18	30/30	1/ND	PR	1
6	0/-	-/ND	4/ND	PR	0.5+ (ongoing)

*Antibody levels day 15 except #1 was day 39, CR, complete remission; PR, partial remission; ND, not determined.

Figure 5. Pharmacokinetics Pt 3/d1



CONCLUSIONS

- SL-401 demonstrates an excellent safety profile in patients with BPDCN.
- A single cycle of SL-401 demonstrates prominent anti-tumor activity in heavily-pretreated patients with advanced BPDCN.
- To date, 83% (5 of 6) BPDCN patients treated with a single cycle of SL-401 had objective responses, with 3 CRs, 2 of which have lasted > 3 mo (1 ongoing at 9+ mo)
- Methods to increase response rate and response duration may include administering multiple cycles of SL-401 and/or administering SL-401 combined with cytotoxic chemotherapy as reported with cytarabine in animal models (Hogge, Leuk Res 28, 1221, 2004). CD123 and CDw13.1 expression will also be evaluated.
- A pivotal program is planned in which SL-401 will be administered in a multiple cycle regimen to patients with advanced BPDCN.
- We acknowledge the support of the University of Texas Southwestern, Scott & White Healthcare, and Stemline Therapeutics for these studies.