ALPN-101 (ICOSL vIgD-Fc), a Dual Antagonist of the ICOS and CD28 Costimulatory Pathways, for Treatment of Steroid Refractory Acute GVHD (aGVHD): Case Report

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Updated Abstract

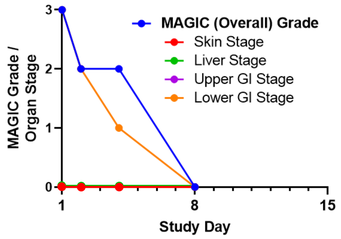
Recent investigations suggest that inhibition of the CD28 pathway by CTLA4-Ig may help prevent or treat aGVHD but responses are often incomplete. ICOS, the most closely related receptor to CD28, and/or its ligand ICOSL, is upregulated during inflammatory responses including aGVHD, and may be an additional pathogenic pathway not addressed when targeting the CD28 pathway alone (Li, 2011). ALPN-101 is an Fc fusion protein of a human ICOSL variant immunoglobulin domain (vIgD™) designed to simultaneously inhibit the ICOS and CD28 costimulatory pathways. In xenogeneic GVHD models, ALPN-101 suppressed acute GVHD and was superior to CTLA4-Ig (2018 TCT abstract #244). In healthy adult volunteers, ALPN-101 was well-tolerated and achieved sustained high levels of target saturation with potent immunomodulatory activity (2020 EULAR abstract #SAT0196). ALPN-101 is in development for the treatment of inflammatory diseases. Herein we report experience for the first GVHD patient who received ALPN-101 (NCT04227938).

A 69-year-old male with a history of successful chemoradiation for stage 4 HPV+ squamous cell carcinoma of the tongue developed secondary myelodysplastic syndrome that failed azacitidine. He received an 11/12 HLA-matched, unrelated peripheral blood stem cell graft after conditioning with fludarabine and melphalan, with neutrophil engraftment by post-transplant day (PTD) 28. Methotrexate and tacrolimus were given as GVHD prophylaxis. The post-transplant course was complicated by severe mucositis, difficult oral intake and aspiration leading to PEG tube placement, and biopsy-confirmed lower intestinal tract GVHD diagnosed on PTD 54 that was refractory to 5 lines of therapy, including high dose methylprednisolone, budesonide and ruxolitinib. Prior to ALPN-101 infusion on PTD 76 (study day 1), he had grade 3 (MAGIC) disease activity, hypoalbuminemia and edema. Other baseline conditions included thrombotic microangiopathy (TMA) ascribed to tacrolimus.

Intravenous infusion of ALPN-101 at 0.3 mg/kg was well tolerated and sustained high level target saturation of circulating T cells for at least 7 days. Stool volume declined within 2 days, permitting steroid taper, and by PTD 83 (study day 8) aGVHD had resolved to grade 0 (Figure 1). Unfortunately, TMA progressed and, despite administration of eculizumab on PTD 79 (study day 3), the patient succumbed to complications of his underlying disease on PTD 86. At the time of death, there was no evidence of aGVHD and methylprednisolone had been tapered from 160 mg to 80 mg daily.

The course suggests that GVHD responded briskly to a single low dose of ALPN-101 following failure of 5 lines of therapy, despite withdrawal of tacrolimus and an abrupt steroid taper, and prior to the potentially confounding addition of eculizumab. Dual inhibition of CD28 and ICOS, such as by ALPN-101, warrants further clinical study for the treatment and/or prevention of GVHD.

Figure 1



Background

The CD28-CD80/86 and ICOS/ICOSL costimulation pathways have emerged as potential therapeutic targets in GVHD, based on efficacy of abatacept administered prophylactically following unrelated stem cell transplant (Watkins 2021) and thalassemia (Khandelwal 2020), and efficacy of ICOS blockade in animal models (Taylor 2005).

CD28-CD80/86 costimulation is essential for full activation of T lymphocytes following initial encounter with antigen. ICOS and/or its ligand ICOSL is upregulated during subsequent inflammatory responses, and may be an additional pathogenic pathway not addressed when targeting the CD28 pathway alone (Li, 2011) (Figure 2).

ALPN-101 is an Fc fusion protein of a human ICOSL variant immunoglobulin domain (vIgD™) that potently inhibits both the ICOS and CD28 costimulatory pathways (Figure 3).

Figure 2

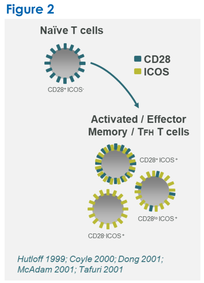
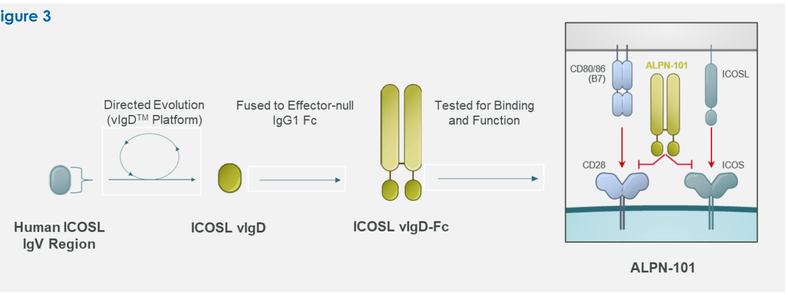


Figure 3



In GVHD models, ALPN-101 suppressed acute GVHD and was superior to inhibition of the CD28 - CD80/86 pathway by belatacept or to inhibition of the ICOS – ICOSL pathway by an unmodified ICOSL-FC fusion protein (Adom, 2020 12:4799; 2018 TCT abstract #244).

In healthy adult volunteers, ALPN-101 was well-tolerated at doses that sustained high levels of target saturation, and exhibited potent immunomodulatory activity, as assessed by inhibition of IgG and IgM response following administration of KLH, and inhibition T cell activation following ex-vivo stimulation (Yang, 2021, in press; NCT03748836).

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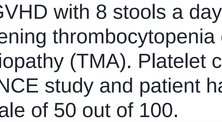
AIS-A02 (BALANCE): ALPN-101 in Steroid-Refractory Acute GVHD (Phase 1b/2)

An open-label study of ALPN-101 in steroid-resistant or steroid-refractory acute GVHD was initiated May 11, 2020 (NCT04227938). Here we report experience with the single subject treated under this protocol.

Study Population
- Adults
- 1st allogeneic stem cell transplant
- Grade 2-4 aGVHD by MAGIC criteria
- Steroid resistant or refractory

Planned Dose Escalation: 3 + 3 Cohorts
- **Starting dose of 0.3 mg/kg administered as a single intravenous infusion was projected to maintain high level receptor occupancy for approximately 1 week**



Selected Study Endpoints
- Safety over 28 days
- PK; target saturation
- Response based on MAGIC criteria; survival
- Use of glucocorticoids

Case Report Summary

A 69-year-old Caucasian male was enrolled for 6th line treatment of MAGIC grade 3 aGVHD involving the lower gastrointestinal system. Medical history and courses of treatment for aGVHD are detailed below.

2016	Stage 4 HPV+ squamous cell carcinoma of the tongue; successfully treated with cisplatin and radiation therapy
2019	High risk secondary myelodysplastic syndrome with complex karyotype requiring transfusions; failed azacytidine, therefore proceeded to allogeneic transplant
Feb 2020	Conditioning with fludarabine / melphalan followed by 11/12 HLA matched unrelated peripheral blood stem cell graft (DPB1 permissive mismatch) on 2/25/20 with tacrolimus and mini-MTX administered for GHVD prophylaxis Neutrophil engraftment by post-transplant day (PTD) 28 PEG feeding required due to mucositis and dysphagia
Mar 2020	Lower intestinal tract GVHD was diagnosed clinically 3/30/2020 (PTD 34) PTD 34: Methylprednisolone 1mg/kg led to initial improvement; diarrhea worsened when steroids were tapered
Apr 2020	PTD 51: Budesonide 9 mg daily and Ruxolitinib 5 mg BID started 4/16/20 PTD 54: Severe GVHD confirmed by biopsy on 4/19/20 PTD 63: Ruxolitinib increased to 10 mg BID 4/28/20 PTD 65: Methylprednisolone increased to 2 mg/kg on 4/30/20 for worsening diarrhea
May 2020	Despite aggressive treatment, patient progressed to MAGIC grade 3 GVHD with 8 stools a day, edema, hypoalbuminemia, and worsening thrombocytopenia concerning for thrombotic microangiopathy (TMA). Platelet count was 47,000 at screening for BALANCE study and patient had a Karnofski Performance Status scale of 50 out of 100.
Enrolled in AIS-02 "BALANCE" Study (PTD 76)	
May 11, 2020 (pre-dose)	Study day 1: Prior to ALPN-101 infusion, patient had MAGIC grade 3 GVHD activity, hypoalbuminemia, and edema. Other baseline conditions included TMA ascribed to tacrolimus, continuing requirement for PEG tube feeding, chronic cough, macrocytic anemia, diabetes mellitus type 2, depression.
May 11, 2020 (treatment)	Study day 1: 0.3 mg/kg of ALPN-101 was administered by intravenous infusion; infusion was well tolerated.
May 18, 2020	Study day 8: Patient achieved remission of aGVHD per MAGIC clinical criteria.

Detailed Course of aGVHD Activity and Treatment Following ALPN-101

- Tacrolimus was stopped 5/13/20 (PTD 78; study day 3) due to concern for thrombotic microangiopathy (platelets 12 x 109 / L, rising LDH, schistocytes on blood smear)
- Eculizimab was started 5/14/20 (PTD 79; study day 4)
- Physical exam and diarrhea improved rapidly after infusion of ALPN-101 and prior to addition of eculizumab, allowing for taper of methylprednisolone from 80mg BID to 80mg QD by 5/18/20 (PTD 83; study day 8)
- The patient achieved remission of aGVHD per MAGIC clinical criteria. Improvement occurred prior to addition of eculizumab, and despite discontinuation of tacrolimus and reduction in dose of methylprednisolone

ALPN-101 Given ↓ Remission noted ↓

Study Day	-1	1	2	3	4	5	6	7	8	9	10	11
		5/11							5/18			5/21
GVHD Disease Activity												
Overall Grade (MAGIC)		3	2		2				0			
Skin		0	0		0				0			
Liver		0	0		0				0			
Upper GI		0	0		0				0			
Lower GI		3	2		1				0			
Key Medications												
Methylprednisolone 80 mg BID -> QD Daily (From Day-11)	160	160	140	140	120	120	120	120	100	100	100	80
Budesonide 9 mg QD (From Day-25)												
Ruxolitinib 10mg BID (From Day-13)												
Tacrolimus BID (From Day-13)												
ALPN-101 0.3 mg/kg												
Eculizumab 900 mg Q1W												
Study Day	-1	1	2	3	4	5	6	7	8	9	10	11

Adverse Events

- Despite improvement in stool output, his course was complicated by worsening TMA with the development of severe thrombocytopenia that predated study drug infusion, anemia and numerous schistocytes requiring cessation of tacrolimus and initiation of eculizumab and platelet transfusions
- Despite initiation of eculizumab, his thrombocytopenia persisted and worsened
- On the evening of Study Day 10, he had acute change in mental status and became less responsive and severely hypoxic. Pupils were not responsive. Platelet counts were noted to be less than 2 x 10^9 / L and he received emergent platelet transfusion. He also became hypotensive and required emergent intubation. He developed sepsis physiology and was started on pressors and broad-spectrum antibiotics
- CT head revealed new subarachnoid hemorrhage
- CT chest revealed dense infiltrates in right lung consisted with aspiration and CT of abdomen and pelvis showed pneumoperitoneum with suspected perforation in the cecum-ascending colon. The small bowel appeared normal. These events were thought to be precipitated by the devastating neurologic injury and considered to be secondary in nature
- Transitioned to hospice in recognition of the profound neurologic injury and succumbed to his underlying disease. Family declined autopsy These serious adverse events were assessed as related to his underlying disease and not related to ALPN-101
- These serious adverse events were assessed as related to his underlying disease and not related to ALPN-101.

ALPN-101 Given ↓ Remission Noted ↓ Acute AMS ↓

Study Day	Screen	1	2	3	4	5	6	7	8	9	10	11
		5/11	5/12	5/13	5/14	5/15	5/16	5/17	5/18	5/19	5/20	5/21
Clinical Adverse Events												
Hypotension – Grade 2			■									
Thrombotic Microangiopathy – Grade 3		■	■	■	■	■	■	■	■	■	■	■
Subarachnoid hemorrhage – Grade 5											■	■
Aspiration pneumonia* - Grade 4											■	■
Sepsis – Grade 4											■	■
Large intestine perforation** - Grade 4											■	■
Laboratory Adverse Events												
Thrombocytopenia	Platelets (normal 134 – 369 x 10E9 / L)	47	35	26	19	12	8	39	30	9	41	11	<2
Anemia macrocytic	Hematocrit (34 – 39.4 %)	25.0	28.9	24.1	21.0	26.7	26.1	24.7	26.8	25.9	24.1	24.2	23.7
Neutrophil count decreased	Neutrophils (1.9 – 7.6 x 10E9 / L)	5.0	7.5	6.6	4.9	3.9	4.1	3.1	2.0	2.7	2.4	0.9	0.2
Blood LDH increased	Lactate Dehydrogenase (100 – 240)	467	567	507	509	521		506		472	479	503	685
Hypoproteinemia	Protein (64 – 82 g / L)	39	44	34		40				41			33
Proteinuria	Urine protein (-)	1+								1+			0
↑ = Albumin given		↑											↑
↑ = Platelets given		↑				↑			↑				↑
↑ = RBCs given		↑		↑									↑

*Chest Radiograph, 5/20/20 showed right upper lobe infiltrate consistent with aspiration

**CT head showed acute subarachnoid hemorrhage

***CT of Chest, Abdomen, Pelvis, showed extensive bilateral pulmonary infiltrates; cholelithiasis; edematous wall thickening suggesting acute cholecystitis; pneumoperitoneum with suspected perforation in the cecum-ascending colon

Summary and Conclusions

- In this patient with severe lower GI acute GVHD refractory to five prior lines of therapy including corticosteroids and ruxolitinib, ALPN-101, a dual inhibitor of CD28 and ICOS achieved a high, sustained level of receptor occupancy on circulating T cells following a single intravenous infusion of 0.3 mg/kg and appeared to reduce GVHD activity within 1 week.
- Stool volume declined within 2 days, permitting steroid taper, and by study day 8 aGVHD had resolved to grade 0. Unfortunately, the patient succumbed to complications of his underlying disease. At the time of death, there was no evidence of active aGVHD and methylprednisolone had been rapidly tapered from 160 mg to 80 mg daily.
- Interpretation is limited by concurrent medications, including ruxolitinib and eculizumab, and by comorbidities, but the clinical course is remarkable for GVHD improvement despite withdrawal of tacrolimus.
- Dual inhibition of CD28 and ICOS, such as by ALPN-101, warrants further clinical study for the treatment and/or prevention of GVHD.