



A Phase I Study of the Toll-like Receptor 5 Agonist, Entolimod, in Patients With Advanced Cancers

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INTRODUCTION

- Toll-like receptor (TLR) family proteins play an important role in pathogen recognition and activation of innate immunity
- They are expressed on antigen presenting cells (APC) and recognize pathogen-associated molecular patterns, like cell wall lipopolysaccharide (LPS), flagella, bacterial and viral nucleic acids
- Once activated, they mobilize nuclear factor kappa B (NF- κ B), interferon regulatory factors (IRFs) and stimulate cytokines production
- Entolimod is a specific TLR5 agonist derived from salmonella flagellin
- Preclinical cancer models have shown immunotherapeutic and radioprotective activities of entolimod
- In healthy volunteers, entolimod has an acceptable safety profile at MTD of 35mcg/day with increases in levels of cytokines, such as G-CSF, IL-6, IL-8, IL-10, and TNF- α within 4 hours following administration and return to baseline by 24 hours
- Here we present a Phase I dose escalation study to determine the MTD, toxicity, pharmacokinetics (PK) and pharmacodynamics (PD) of entolimod in patients with advanced cancers

OBJECTIVES

Primary Objective:

- Determine the safety and the maximum tolerated dose (MTD) of Entolimod

Secondary Objectives:

- Assessment of preliminary evidence of efficacy in patients with advanced cancer
- Evaluate pharmacokinetic (PK) and pharmacodynamics (PD) profiles

Exploratory Objectives:

- To correlate pre-treatment tissue expression of TLR5 with clinical activity (RECIST tumor response)
- To correlate single nucleotide polymorphisms (SNPs) in the TLR5 gene with toxicity and clinical outcome

ELIGIBILITY CRITERIA

- Patients with histologically or cytologically confirmed locally advanced, inoperable or metastatic solid tumor for which no acceptable therapy exists
- 18 years of age or older
- ECOG Performance Status of 0 or 1
- Life expectancy greater than 3 months
- Adequate bone marrow, liver, cardiac and renal functions
- No previous exposure or hypersensitivity to entolimod

METHODS

- Escalating doses of entolimod were administered in the traditional "3 + 3" dose escalation scheme (Table 1)
- Safety assessment included vital signs, ECOG performance status, physical examination and laboratory tests
- Adverse events were defined and graded according to CTCAE (v.4.0)
- DLTs were defined as any AEs related to entolimod, and were evaluated within the first 21 days of treatment
- Tumor assessment was per revised RECIST (v.1.1) criteria at baseline and at 6 weeks interval or as clinically indicated
- A population PK model was used to estimate AUCs and CL
- PD analysis was for cytokine levels, antibody production and immune assessment by flow cytometry
- Genotype analysis for the common polymorphism in TLR5

Table 1. Dose escalation scheme

Dose Level	N. of Patients (total=26)	Entolimod Dose
1 (starting dose)	4	5 mcg/day (daily for 5 days)
2	3	10 mcg/day (daily for 5 days)
3	3	15 mcg/day (daily for 5 days)
4	4	20 mcg/day (daily for 5 days)
5	5	30 mcg/day (day 1, 4, 8, and 11)
6	7	40 mcg/day (day 1, 4, 8, and 11)

RESULTS

Patients were enrolled from January 2012 to October 2014 and received treatment at RPCI (Table 2)

Safety

- Treatment was well tolerated
- The MTD dose was determined to be 30 μ g/day
- At DLT dose (40 μ g/day): One patient developed grade 3 rigors, pyrexia and prolongation of QTc interval; a second patient had grade 3 trnasaminitis; a third had grade 3 hypotension
- Treatment related AEs (CTCAE grade 3 or higher):

	30 mcg/day (N=5)	40 mcg/day (N=7)	Total
ECG QTc	0	1	1
trnasaminitis	1	1	2
Pyrexia	0	1	1
Hypotension	2	1	3
Rigors	0	1	1

Table 2. Patients Baseline Characteristics

Characteristics	No. (N=26)	%
Age, years		
Range	42-82	
Median	64	
Sex		
Male	17	65.4
Female	9	34.6
Race		
Caucasian	22	84.6
Black	3	11.5
Asian	1	3.8
Previous Treatment		
1-2	4	15.4
≥ 3	22	84.6
Primary Tumor		
colorectal cancer	10	38.5
NSCLC	5	19.2
Bladder	2	7.7
Other*	9	34.6
ECOG PS		
0	3	11.5
1	23	88.5

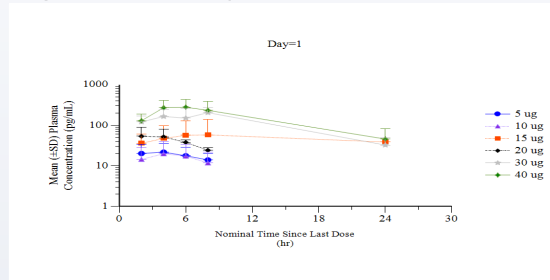
Common adverse events are as below:

Adverse Events	Grades				Total N (%)
	1	2	3	4	
Injection site reaction	18	0	0	0	18 (69.2)
Palpitation	15	0	0	0	15 (57.7)
Rigors	10	3	1	0	14 (53.8)
Pyrexia	10	3	1	0	14 (53.8)
Headache	7	0	0	0	7 (26.9)
lymphopenia	0	2	1	1	4 (15.4)
QTc prolongation	7	3	1	0	11 (42.3)
Hyperglycemia	8	6	0	0	14 (53.8)
Trnasaminitis	12	0	2	0	14 (53.8)
Hypertension	3	4	1	0	8 (30.8)
Hypotension	9	7	3	0	19 (73.1)

Pharmacokinetics

- Entolimod PK appears dose proportional and follows a monoexponential decay
- PK profiles on days 4, 8, and 11 are comparable to day 1, demonstrating no accumulation following multiple doses of entolimod (Figure 1)

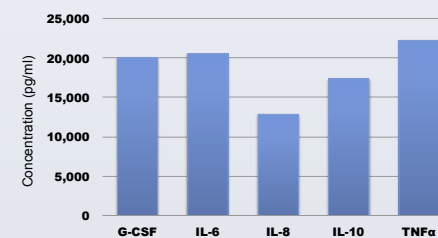
Figure 1: PK Summary



Pharmacodynamics

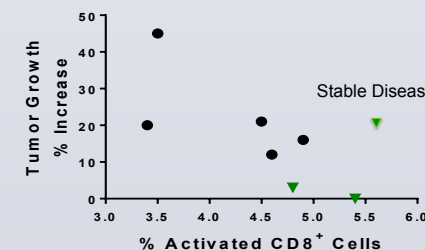
- Cytokines surge was 4-6 hours after the first dose in all treated patients and returned to baseline level within 24 hours (Figure 2)

Figure 2: Induced cytokines



- Patients exhibited immune cell activation with a stable or decrease in the levels of suppressive immune cells
- Activated CD8⁺ T cells were defined by expression of CD3, CD8 and perforin (Figure 3)

Figure 3. Activation of CD8⁺ T cells following entolimod injections correlates with tumor growth



- 18 patients were evaluable for tumor response (4 withdrew consent and 4 discontinued due to DLT)

Table 3: Efficacy - Stable disease achieved in 8 patients at 6 wks

Dose	Tumor Type	Disease Status	6 weeks	12 weeks
5 mcg/day	colon cancer	SD		PD
10 mcg/day	peritoneal mesothelioma	SD		PD
15 mcg/day	urothelial carcinoma	SD		PD
15 mcg/day	metastatic melanoma	SD		PD
15 mcg/day	urothelial carcinoma	SD		SD ¹
20 mcg/day	SCC of anus	SD		SD
30 mcg/day	gastric adenocarcinoma	SD		PD
40 mcg/day	colorectal cancer	SD		SD ²

SD: Stable Disease; PD: Progressive Disease; SCC: Squamous Cell Carcinoma
1: Patient removed from study due to continued antibodies elevation 2: stable disease >18 weeks.

Pharmacogenetics

TLR5 SNPs Associated with Adverse Events								
Event Category	dbSNP id	Location and HGVS Names	Study MAF	Global MAF	Genotype (N)	N (%) Events	Odds Ratio (95% CI)*	P-value
LFT elevation	rs1028854 A>G	Exon 5; synonymous codon; c.2523A>G; p.Lys841=	0.135	0.0857	AG+GG (6) AA (20)	6 (100%) 5 (25%)	36 (1.8, 763)	0.002
	rs1744157 C>G	Intron, c.-188-955C>G	0.115	0.0369	CG+GG (5) CC (21)	5 (100%) 6 (29%)	36 (1.3, 546)	0.007
	rs2240977 A>G	Intron, c.-5-104T>G	0.288	0.1689	TG+GG (13) TT (13)	9 (69%) 2 (15%)	12 (1.8, 83)	0.015

¹ Genotypes are shown with Global minor allele from 1000 Genome phase 1 data of 1094 worldwide individuals (May 2011 dataset)
² CIs are based on logit transformation (and add a constant, 0.5, to all cells for tables with zero cells)
³ Unadjusted P-value from Fisher's exact test

CONCLUSION

- Entolimod was well tolerated
- The recommended phase II dose of Entolimod is 30 μ g/day on days 1, 4, 8 and 11
- Plasma pharmacokinetics appear generally dose proportional and follow a mono-exponential decay
- The clinical results corroborated pre-clinical findings and support entolimod's potential as an immunotherapeutic agent
- The safety profile suggests that entolimod can be combined with chemotherapeutic, targeted, or other immunotherapeutic agents