Update on the Novel Prodrug Dual mTOR-PI3K Inhibitor SF1126

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Abstract

The phosphatidylinositol 3-kinase (PI3K) pathway is one of the most commonly activated pathways in human cancer and has roles in cell proliferation, apoptosis, protein synthesis and metabolism. The PI3K pathway can be activated by amplification or activating mutation of upstream receptor tyrosine kinases, and by mutations or deletions downstream in the pathway. Because of the common activation of the PI3K pathway in cancer, compounds targeting proteins downstream in the pathway have been developed in recent years. The mTOR inhibitors everolimus and temsirolimus have been shown to be beneficial in certain cancer types; many other inhibitors of the PI3K pathway are in various stages of clinical development.

LY294002, a small molecule that inhibits all four class I PI3K isoforms along with a select number other cancer targets such as mTOR, DNA-PK, PIM1, and PLK1, has been one of the most successful and most widely used pathway inhibitors in research. Independent of its kinase inhibition mechanism of action it can also induce oxidative stress in cancer cells. In view of the fact that LY294002 suffers from poor bioavailability, conjugation to an integrin-targeting peptide resulted in the pro-drug SF1126, which increased solubility and also enhanced tumor delivery of the active inhibitor in xenograft models.

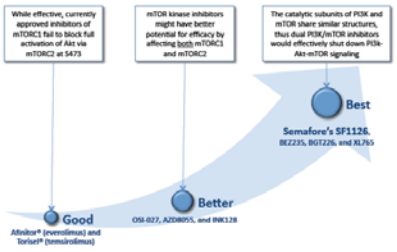
SF1126 is in development in multiple phase I clinical trials as a single agent and interim results have been presented recently in patients with solid tumors (ASCO, 2009, abstract 2558) and multiple myeloma (ASH, 2009, abstract 3879). While SF1126 is not as potent in its kinase inhibition as other clinical stage PI3K inhibitors its *in vivo* activity compares favorably perhaps due to the unique vascular targeting potential along with the inhibition of multiple kinase targets unique to SF1126.

In an ongoing phase I dose escalation study in patients with solid tumors SF1126 is administered days 1, 4 weekly by 90 minute IV infusion in cycles of 4 weeks. To date 18 of 39 (46%) of dosed patients showed stable disease as best response with a median duration of 13 weeks (range 8- 64+) and a mean duration of about 19 weeks. Of particular note is a renal cell carcinoma patient previously resistant to sunitinib, sorafenib, temsirolimus and interferon/bevacizumab with stable disease for 16 cycles and is still on study representing possible evidence of abrogating MTORC1 resistance. In this study the MTD was not reached with the maximum administered dose being 1110 mg/m^2. SF1126 is well tolerated with the most common grade 1 adverse events being nausea, vomiting, diarrhea, fever, fatigue, chills and pruritus. The PK appears dose proportional and exposure levels achieved at ≥ 140mg/m^2 exceeded levels found to be effective in preclinical xenograft studies.

Recent preclinical work (AACR, 2010) along with literature reports provided support for expanding the phase I single agent clinical trials of SF1126 into B-cell malignancies such as CLL and mantle cell lymphoma. Preliminary results of SF1126 clinical trial expansion into B-cell malignancies will be presented.

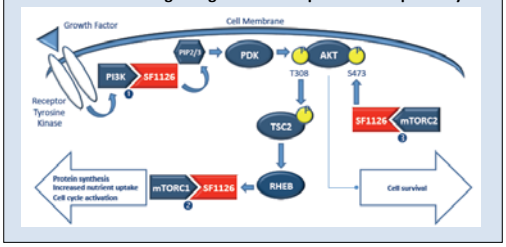
Lastly, the experience in PI3K inhibitors has been leveraged into a platform for next generation kinase inhibitors that are designed to simultaneously inhibit multiple pathways with a single small molecule. This platform is designated CRIMP (Control Resulting from Inhibition of Multiple Pathways) and has resulted in SF2626, the first small molecule demonstrated to inhibit both MEK (MAPK pathway) and PI3 kinase.

Evolution of PI3K Pathway Inhibition



Structure and Function of SF1126

SF1126 is a dual PI3K/mTOR inhibitor that effectively shuts down PI3k-Akt--mTOR signaling at 3 distinct points in the pathway

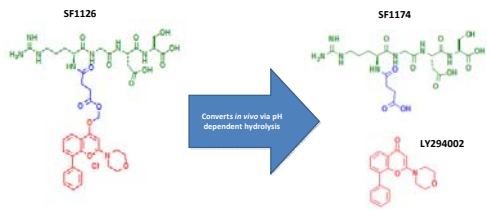


PRODRUG CONJUGATE DESIGN:

1. Allows the concentration of the active drug to build to high levels in plasma for an additional tumor exposure in a way that decreases toxicity
2. Gets more active drug in the vicinity of the tumor using an integrin receptor antagonist to help contribute to overall efficacy (demonstrated in animal models: Cancer Res. 2008 vol.68, p208)

SF1126 PRODRUG CONJUGATE CONTAINS THREE COMPONENTS:

1) Integrin antagonist
   - –SF1126 targets integrins, such as αvβ3, via an arginine-glycine-aspartic acid (RGD) peptide
   - –Integrins are transmembrane receptors critically involved in many tumor-promoting activities, such as proliferation, survival, invasion, and angiogenesis
2) Cleavable Linker
   - –Linker is cleaved due to pH dependent hydrolysis (more stable at low pH)
   - –pH = 7.4, conversion half life around 1 hour
3) Unique kinase inhibitor (LY294002)
   - –Pan PI3K/mTOR inhibitor along with other important cancer targets
     - LY294002 is one of the most widely studied PI3K/mTOR inhibitors
     - Subject of nearly 5,000 scientific publications
     - Poor bioavailability and potential acute toxicity if administered alone (without linker and targeting moiety)



SF1126 INHIBITION PROFILE:

SF1126 inhibits all four PI3K Class I isoforms (optimal for cancer therapy)
– IC50 values:

PI3K alpha:	356nM
PI3K beta:	736 nM
PI3K delta:	3225 nM
PI3K gamma:	1774 nM

SF1126 also inhibits other important kinase targets
– IC50 values:

mTOR kinase;	1060 nM
DNA-PK:	357 nM
PIM1:	830 nM
PLK1:	1900 nM

SF1126 has additional anticancer properties independent of PI3K inhibition
– Creates intracellular hydrogen peroxide = oxidative stress
– Sensitizes LNCaP cells to low doses of vincristine
– Results in apoptosis

Despite better in vitro potency from other inhibitors versus Semafore's released inhibitor LY294002…

Company	Compound	PI3K-α	PI3K-β	PI3K-δ	PI3K-γ	mTOR	DNA-PK
Semafore	LY294002	356	736	3225	1774	1060	357
Novartis	BEZ235	4	76	7	5	20	n/a
Exelixis/Sanofi	XL147	39	383	36	23	n/a	4750
Exelixis/Sanofi	XL765	39	113	43	9	157	150
Roche	GDC0941	6	38	4	80	581	n/a

SF1126 compares favorably in mouse models at lower doses and less frequent dosing:

Company	Compound	Tumor Type	Dose (mg/kg)	Route	Doses per wk	Outcome (% TGI inhibition)	Days post dosing
Semafore	SF1126**	U87MG	12	i.v.	5	105	15
Semafore	SF1126**	U87MG	12	i.v.	3	86	14
Novartis	BEZ235	U87MG	45	p.o.	7	88	10
Roche	GDC0941	U87MG	75	p.o.	7	100	22
Semafore	SF1126**	PC3	20	i.v.	3	80	14
Roche	GDC0941	PC3	75	p.o.	7	75	12
Roche	GDC0941	PC3	75	p.o.	14	94	12
Exelixis/Sanofi	XL147	MDA-MB-468	100	p.o.	7	74	17
Semafore	SF1126**	MDA-MB-468***	25	i.p.	3	47	26

** Doses of SF1126 are in mg/kg of active PI3K inhibitor contained therein: multiply by 3 to get total mass dosed
*** Orthotopic model; all others are s.c. xenograft mouse models

SF1126 Phase I Solid Tumor Clinical Trial

PHASE 1 OBJECTIVES, DESIGN AND PATIENT SELECTION:

- **Objectives**
  - Identify Phase 2 dose
  - Determine safety profile
  - Characterize pharmacokinetics of SF1126 and major metabolites
  - Determine effect on pharmacodynamic markers
    - Tumor and normal tissue (skin)
  - Assess antitumor response
- **Design**
  - Administration: BIW via 90 min. IV infusion in 4 week cycles
  - Escalation: standard, modified Fibonacci escalation
  - Cohorts: one patient per cohort until Grade ≥ 2 toxicity, then 3 pts / cohort
- **Patients**
  - Refractory solid tumors in adults ≥ 18 yrs age
  - ECOG 0-1
  - Emphasis on tumor types associated with PI3K pathway abnormalities

PATIENT DEMOGRAPHICS

Diagnosis		
Breast	3	8%
CRC	10	26%
Endometrial	1	3%
GIST	7	18%
NSCLC	1	3%
Ovarian	5	13%
Pancreatic	2	5%
Prostate	3	8%
RCC	3	8%
Sarcoma	1	3%
SCCHN	1	3%
Unknown	1	3%
Urachal	1	3%
TOTAL:	**39**	

Demographics and Baseline Characteristics	
Age	59 (24-84)
Gender	
Male	15 (38%)
Female	24 (62%)
ECOG Performance Status	
0	20 (51%)
1	19 (49%)
Prior Chemotherapy Regimens	
Number	4 (1-14)

SF1126 PHASE I SAFETY SUMMARY

1) Well tolerated (most reactions Grade 1/2)
2) Most common adverse events were nausea, vomiting, diarrhea, fever, and fatigue (infusion reactions minimal and manageable)
3) No clinically significant changes reported in glucose or insulin

SF1126 PHASE I PHARMACOKINETICS SUMMARY

- Targeting moiety of SF1126 (arginine-glycine- aspartic acid peptide)
  - Cmax and AUC_{0-t} tended to increase with increasing dose
  - C_{max} at top dose >15 μM
  - Mean $t_{1/2}$ about 1 hour
- Active PI3K/mTOR inhibitor (LY294002)
  - C_{max} and AUC_{0-t} dose proportional
  - Mean C_{max} at top dose >20 μM
  - Mean $t_{1/2}$ for dose groups ranged from 1.1 to 2.2 hr
  - AUC_{0-t} values at doses ≥ 140 mg/m^2 exceed exposures found efficacious in mouse studies

SF1126 PHASE I: DOSING, DLTS AND CLINICAL ACTIVITY

Cohort	Dose mg/m^2	Patients Dosed	Patients Completed ≥ 1 cycle (DLT Evaluable)	DLT	Other Grade 3/4 Toxicities	Patients on Study ≥ 2 Months	Patients with Stable Disease	Duration (weeks)
1	90	6	3		Diarrhea (1)*	1	Prostate	20
2	140	3	3			1	Breast	8
3	180	6	6	Diarrhea (1)*		3	Endometrial GIST Ovarian	20 20 12
4	240	3	3			2	GIST CRC	12 8
5	320	3	3			2	Pancreas CRC	15 12
6	430	4	3			3	GIST Ovarian CRC	12 12 13
7	630	3	3		Urticaria & Pruritis (1)+	3	CRC CRC RCC	12 13 14
8	840	3	3			3	RCC	64+
9	1110	8	6			6	Ovarian TUO GIST	26 16 40+
Total		39	33			24	19	Median 13 Mean 18

*same patient, initial dose 180 then lowered to 90 mg/m2 and retreated
+single patient with grade 3 urticaria and pruitis in cycle 3, not DLT

SF1126 PHASE I CLINICAL ACTIVITY SUMMARY

- Efficacy Summary
  - 19 of 39 (49%) dosed patients (19 of 33 (58%) evaluable patients) showed stable disease as best response with a median duration of 13 weeks (range 8-64+); mean 18 weeks
- Additional signs of clinical activity:
  - RCC patient stable on SF1126 for >16 Cycles (64 weeks) and still on study
    - Prior treatments:
      - Progressed on sunitinib at 2 mo
      - Progressed on sorafenib at 1 mo
      - Progressed on interferon/bevacizumab at 3 mo
      - Stable on temsirolimus for 15 mo
    - Possible evidence of effect of SF1126 on mTORC1 resistance
  - CRC patient requiring regular paracentesis for ascites had markedly decreased paracentesis while on study
  - 60 year old male with metastatic PCA experienced decreased bone pain for 26 wks

SF1126 Phase I Clinical Trial Expansion Into Patients with B-Cell Malignancies

PRELIMINARY CLINICAL SUMMARY OF TWO CLL PATIENT TREATED TO DATE:

Patient 1:
17p Deletion with no prior treatments
Entered study with WCC doubling every 3 months
Initial flare noted C1D1/C1D4 timeframe in lymph nodes
Remarkable decrease in size of lymph nodes (C1D8/C1D11 timeframe)
Significant drop in WCC (40%) initially followed by fluctuations
No DLTs

Patient 2:
Heavily pretreated (5 previous lines of therapy)
Initial flare noted C1D1/C1D4 timeframe in lymph nodes
WCC fluctuating
Additional PD tests in progress
No DLTs

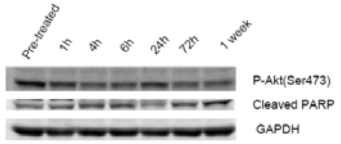
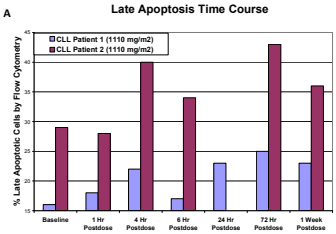


Figure 1. Western blot analyses of isolated lymphocytes from the first CLL patient treated at 1110 mg/m^2 demonstrate decreased pAKT (473) signaling and increased PARP cleavage over time relative to baseline.

A



B

	CLL Patient 1 (1110 mg/m2)	CLL Patient 2 (1110 mg/m2)
1 Hr Postdose	13%	-3%
4 Hr Postdose	38%	38%
6 Hr Postdose	6%	17%
24 Hr Postdose	44%	No sample
72 Hr Postdose	56%	48%
1 Week Postdose	44%	24%

Figure 2. A) Time course analysis by flow cytometry of isolated lymphocytes from the first two CLL patients treated at 1110 mg/m^2 demonstrate consistent increases in late apoptosis over time relative to baseline following 1st SF1126 dose; B) Same data tabulated as percent increases in late apoptosis from baseline

Overview: PI3K Inhibitors in Phase I Clinical Trials

	SF1126 Semafore	XL765 Exelixis-Sanofi	XL147 Exelixis-Sanofi	GDC-0941 Roche (GDC4254g study)	GDC-0941 Roche (GDC4255g study)	GDC-0980 Roche (PIM4604g study)	PX-866 Oncothyreon	BEZ235 Novartis	BKM120 Novartis	CAL-101 Calistoga
Target	PI3K/mTOR	PI3K/mTOR	PI3K	PI3K	PI3K	PI3K/mTOR	PI3K (irreversible)	PI3K/mTOR	PI3K	PI3K delta
# pts	39	83	78	31	40 (QD) 27 (BID)	17	51 [9]	59	35	99 (BID) 7(QD)
Admin. route	IV	Oral	Oral	Oral	Oral	Oral	Oral	Oral	Oral	Oral
Schedule (all 28 day cycles)	4wk on	4 wk on	3wk on/1wk off [also CDD]	3wk on/1wk off	3wk on/1wk off	3wk on/1wk off	2wk on/2wk off [also CDD]	4 wk on	4 wk on	4 wk on
Frequency	BIW	BID, QD	QD	QD	QD, BID	QD	QD (5 /7 days) [QD]	QD	QD	BID, QD
MTD (or Max Admin. Dose)	1110 mg/m2 (MAD)	BID: 50mg (120 MAD) QD: 90mg (100 MAD)	600mg (900 MAD) [600 mg]	245mg (MAD)	QD:330mg (MAD) BID: 245mg TDD (MAD)	32 mg (MAD)	12 mg (16 MAD) [8 mg (10 MAD)]	1100 mg (MAD)	100 mg (150 MAD)	BID: 150 mg (350mg MAD) QD: 300 (MAD)
DLTs	Diarrhea	BID: Rash, nausea, vomiting, hyperphosphatemia ,hyponatremia, transaminases QD: abnormal tCO2, rash/fatigue, dyskinesia	Rash [hypersensitivity]	None	Headache, pleural effusion, ↓ DLCO	None	Diarrhea, AST [diarrhea]	None	Mood alterations, epigastralgia , rash, hyperglycemia	↑AST/ALT
5 Most Frequent AEs in descending order *(italics are tied for frequency)*	Nausea, vomiting, diarrhea, fever, fatigue	Nausea, diarrhea, ↓ appetite, vomiting, ↑trans-aminases	Nausea, fatigue, diarrhea, rash, vomiting, *↓appetite*	Nausea, diarrhea, dysgeusia, vomiting, *fatigue, ↑AST or ALT*	Nausea, dysgeusia, diarrhea, fatigue, rash	Diarrhea, fatigue, ↓ appetite, *constipation, flatulence,* nausea, abd *pain, oral pain, rash*	Diarrhea, nausea, vomiting, constipation, *fatigue, anorexia* [Diarrhea, ↑ALT or AST, *nausea, fatigue, headache,* vomiting]	Fatigue/ asthenia, diarrhea, nausea, vomiting, anorexia	Rash, hyperglycemia, diarrhea, anorexia, nausea	≥Grade 3 AEs: ↑AST/ALT, pneumonia, neutro-penia, anemia, thrombo-cytopenia [↑AST/ALT, pneumonia]
Efficacy (reported *or calculated*)	17 pts (52%) ≥ 3 months SD; 7 pts (21%) ≥ 4 cycles SD	12 pts *(14%)* ≥ 4 cycles SD	1 PR (NSCLC) 14 pts (19%) ≥ 4 cycles SD	5 pts *(16%)* ≥ 3 cycles SD	1 PR (breast) 14 pts (21%) ≥ 3 cycles SD	4 pts *(23%)* ≥ 4 months SD	7 pts(16%) ≥ 4 cycles SD [3 (38%) ≥ 4 cycles SD]	2 PRs (breast, lung/ Cowden), 14 (27%) ≥ 4 cycles	1 PR (breast) 13 pts (42%) >6 wks SD	ORR: Indolent NHL 57%, MCL 67%, CLL 30%, AML 0%, MM 0%, DLBCL 0%
Source	Company	'10 ASCO #3030	'10 ASCO #3004	'10 ASCO #2613	'10 ASCO #2541	'10 ASCO #3079	'10 ASCO #3089	'10 ASCO #3005	'10 ASCO #3003	'10 ASCO #3032; '09 ASH #922

CONCLUSIONS

Pathway Evolution
- It is well established that currently approved mTORC1 inhibitors, such as everolimus and temsirolimus, often lead to a feedback activation of PI3K in cancers
- Dual PI3K/mTOR inhibitors might therefore mitigate this feedback activation of PI3K signaling and yield greater therapeutic benefit

Structure and Function of SF1126
- Several strategies have been used to design prodrugs of various poorly absorbed drugs targeted to receptors and transporters for improved bioavailability, such as paclitaxel albumin-bound particles (Abraxane® by Abraxis BioScience, LLC)
- In this connection, SF1126 is a peptidic prodrug that converts to LY294002, one of the most widely studied dual PI3K/mTOR inhibitors that historically suffered from poor solubility
- LY294002 is conjugated to an Arg-Gly-Asp (RGD) peptide via a pH cleavable linker to form SF1126, which leads to increased solubility and better tolerability
- As a prodrug with improved properties and site selectivity due to targeting to RGD-recognizing integrin receptors, SF1126 opened up a new avenue for the clinical development of LY294002
- As the only PI3K/mTOR inhibitor prodrug currently in development, SF1126 is designed to accumulate preferentially in tumor tissue in an attempt to maximize efficacy and minimize toxicity
- *In vivo* efficacy studies showed that SF1126 has improved activity compared with LY294002
- SF1126 has the broadest spectrum of desirable kinase target inhibition which may be best taken advantage of in combination therapy
- Despite modest enzyme-level potency SF1126 compares favorably in dose levels required to produce efficacy in animal models versus other more potent clinical stage PI3K inhibitors
- SF1126, a peptide prodrug of LY294002, may constitute a significant therapeutic advantage in the treatment of cancer by inhibiting the PI3K/mTOR pathway through preferential accumulation in tumor tissue

SF1126 Phase I Solid Tumor Trial
- SF1126 is very well tolerated at high dosing, not reaching an MTD in single agent Phase I trials
- SF1126 demonstrates prolonged clinical control of disease in a heavily pretreated solid tumor population, including evidence of activity in an mTORC1 resistant RCC patient with stable disease for more than 64 weeks
- The lack of toxicity of SF1126 at 1110 mg/m^2 bodes well for combining with other cancer therapies

SF1126 Phase I B-Cell Malignancy Expansion
- Initial preliminary results at the MAD (1110 mg/m^2) without toxicity demonstrate measurable desirable changes in disease status supporting pan-PI3K inhibitor utility in hematological disease.

Clinical Stage PI3K Inhibitor Comparisons
- PI3K inhibitors show various degrees of stable disease in solid tumors with occasional partial responses; significant numbers of disease-specific partial responses were observed in certain hematological malignancies
- Toxicities such as nausea, vomiting, diarrhea, and fatigue appear to be a class effect with surprisingly few reports of hyperglycemia
- Liver function toxicities resulting in DLTs were noted with 3 of the 8 oral inhibitors perhaps related to the oral route of administration since none were seen with i.v.-dosed SF1126

SEMAFORE PHARMACEUTICALS