The Protein Experts
Cytoskeleton, Inc.

Custom Services Newsletter

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Quarter 3, 2014
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KSP (Eg5) Inhibition – Theory and Experimental Studies

The Kinesin Spindle Protein (KSP; also known as Eg5 or KIF11) is a Kinesin-5 subfamily member and has been the focus of a significant drug development effort throughout the pharmaceutical industry for the last 15 years. KSP plays a critical role in mitotic spindle pole separation, and its inhibition results in the formation of monoaster spindles which is thought to lead to mitotic catastrophe and apoptosis[1]. From a therapeutic standpoint, it was anticipated that inhibiting KSP would produce antimitotic benefits by targeting microtubules with fewer and less severe side effects. Microtubule targeting drugs (e.g., Paclitaxel and Vincristine) are quite efficacious and continue to be a front line therapeutic approach for several cancers (e.g., ovarian, breast, gastroesophageal)[2]. These drugs, however, are associated with significant and occasionally debilitating side effects[2,3]. Although microtubules play a critical role in chromosomal segregation during mitosis, they also have important roles in postmitotic cells like neurons where vesicle transport can be disrupted by microtubule-targeted drugs, leading to peripheral neuropathies[2,3]. The original identification of the KSP inhibitor monastrol in 1999 by Mayer et al[4] opened the door to a promising new direction for antimitotic drug discovery. Monastrol itself did not exhibit the appropriate drug-like characteristics necessary to be tested in human clinical trials; however, a growing list of second generation KSP inhibitors have been developed with improved pharmacokinetic properties.

Clinical Trials with KSP (Eg5) Inhibitors

In 2004, Ispinesib (SB-715992), developed by Cytokinetics and GlaxoSmithKline, became the first KSP inhibitor to enter clinical trials[5]. There have been 35 Phase 1/2 clinical trials initiated to date with 8 chemically distinct KSP inhibitors (see Table 1). The preponderance of evidence suggests that KSP inhibitors are well-tolerated and as predicted, they do not exhibit the neurotoxicities associated with microtubule-targeted drugs[6]. KSP inhibitors target rapidly dividing cells and consequently they do share dose-limiting toxicities with other antimitotic therapies, including neutropenia and leukopenia (lowered neutrophil and leukocyte levels, respectively)[6]. Unfortunately, many of these KSP inhibitors have failed to show efficacy in a clinical setting as a monotherapy. Array BioPharma's Filanesib (ARRY-520) has been the exception as it has demonstrated efficacy as a monotherapy for the treatment of multiple myeloma[7]. Filanesib is slated to be the first KSP inhibitor to be used in a Phase 3 clinical trial and will be tested as a monotherapy and in combination with standard chemotherapeutic agents[8].

Some of the more recent preclinical studies using KSP inhibitors suggest that therapeutic synergy is possible when KSP inhibitors are combined with other chemotherapeutic agents[9]. This could potentially allow lower levels of each drug to be used, which could lead to fewer and less pronounced side effects. If these results translate into the clinical setting, we may be on the verge of seeing significant clinical benefits with KSP inhibition combo therapies.

Cytoskeleton Custom Services – Antimitotic Drug Candidates

At Cytoskeleton, we have developed assays for 11 recombinant kinesin motor domains that represent 8 of the 14 recognized Kinesin subfamilies[10]. We offer compound screening services with individual kinesins as well as multi-motor protein panel studies. Moreover, if the kinesin assay needed for your research is unavailable, we offer custom protein expression/purification and assay development services to help move your project forward. In addition to kinesin assays, we can evaluate your compounds for their effects on microtubule polymerization. This may be a useful tool in combination with our kinesin panel screen to identify the mechanism of action for antimitotic compounds coming from phenotypic screens and/or as a useful counterscreen for kinesin inhibitor drug discovery efforts that desire to steer their SAR efforts away from compound effects on tubulin polymerization.

Table 1: KSP (Eg5) Inhibitor Clinical Trials

Generic Name	Alternate Name	Company	Phase 1	Phase 2	Phase 3
Ispinesib	SB-715992	Cytokinetics & GSK	5	5	
Filanesib	ARRY-520	Array BioPharma	3	4	1*
Litronesib	LY2523355	Eli Lilly & Co.	4	3	
	AZD4877	AstraZeneca	5	1	
	SB-743921	Cytokinetics	1	1	
	MK0731	Merck Sharp & Dohme Corp.	1		
	4SC-205	4SC	1		
	ARQ621	ArQule	1		

Data source = www.clinicaltrials.gov
*Planned initiation mid-2014[8]

References

1. Sarli V. and Giannis A. 2008. *Clin. Cancer Res*. **14**, 7583-7587.
2. Morris P.G. and Fornier M.N. 2008. *Clin. Cancer Res*. **14**, 7167-7172.
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6. Knight S.D. and Parrish C.A. 2008. *Curr. Topics Med. Chem*. **8**, 888-904.
7. Shah J.J., Zonder J., Cohen A. et al. 2011. *Blood* (ASH Annual Meeting Abstracts). **118**, 1860.
8. Owens B. 2013. *Nat. Med*. **19**, 1550.
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Custom Modules

Our recently expanded Custom Services Department provides additional resources for your research projects.

Cytoskeleton is leading the way to develop novel kinesin, dynein, and myosin based compound screens.

We are scientists dedicated to providing accurate data reported in a detailed and timely manner.

About Custom Services

Like our regular product offerings, the Custom Services Department emphasizes quality products and services. We understand that **accuracy** and **timeliness** are critical elements for a successful project. Choose from more than twenty defined modules (**for a full list, visit www.cytoskeleton.com/custom-services**), and then contact Technical Support (tservice@cytoskeleton.com) to guide you through the process.

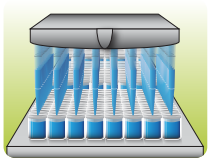
Clients Include:

- Merck & Co., Inc.
- Eli Lilly & Co.
- Amgen, Inc.
- Abbott Laboratories
- Pfizer, Inc.
- Astra-Zeneca plc
- GlaxoSmithKline plc
- Genentech, Inc.
- Johnson & Johnson
- Bristol-Meyers Squibb

Let's get started, it's as easy as 1,2,3 ...

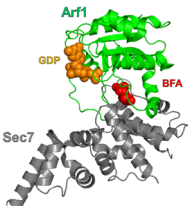
1. Choose a module and ask for a quote (24h turn around time)
2. Review quote, specifications, and deliverables
3. Place order and receive regular updates until project is finished

Compound Screening Modules



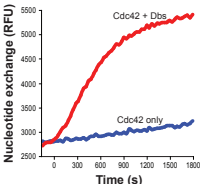
Type	Format	Deliverable	Module #	Timeline (wks)
Eg5 Kinesin motor assay	Microtubule stimulated ATPase assay, kinetic, absorbance at 360nm	96 assays, consisting of 40 duplicate single concentrations (or 5 x IC50s), plus eight control wells. PDF Report with Executive Summary, Introduction, Methods, Results and Data Analysis.	CDS050 or CDS051	2
Cardiac Myosin motor assay	Ca^{2+}/Sarcomere (thin filament) stimulated ATPase assay, kinetic, absorbance at 360nm	Same as CDS052.	CDS056	2
Dynein motor assay	Microtubule stimulated ATPase assay, kinetic, absorbance at 360nm	Same as CDS052.	CDS065	2
Tubulin polymerization	Tubulin (>99% pure) Polymerization Assay, kinetic, fluorescence at 360nm/410nm	96 assays, with 40 duplicate single concentrations or 5 x IC50s, plus eight control wells (vinblastine, nocodazole or taxol). PDF Report with Executive Summary, Introduction, Methods, Results and Data Analysis.	CDS009 or CDS010	2
GEF/GTPase exchange assay	GTP exchange factor plus Small G-protein (e.g. Rho or Ras) with mant-GTP reporter. Kinetic, fluorescence at 360nm/450nm	60 assays consisting of either 28 duplicate reactions plus 4 controls, or 5 x IC50s plus 1 x control IC50. PDF report with Executive Summary, Introduction, Methods, Results and Data Analysis.	CDS100	2

Gene Cloning and Protein Purification Modules



Type	Name	Deliverable	Module #	Timeline (wks)
Recombinant Small Protein	Small protein or protein domain (<30 kDa) with gene provided by client	Highly purified, His-tagged active protein lyophilized in 10 x 100 µg aliquots (or more depending on yield). Datasheet and assay method. Activity in line with published articles. *E. coli* expression.	REC012	3
Recombinant Small Protein plus cloning	Small protein or protein domain (<30 kDa) including gene synthesis	Same as above with gene synthesis.	REC022	6
Recombinant Kinesin Motor-Protein	Medium to large protein or protein domain (30-100 kDa)	Same as REC012.	REC032	3
Recombinant Kinesin Motor Protein plus gene cloning	edium to large protein or protein domain (30-100 kDa) with gene synthesis	Same as above with gene synthesis.	REC042	8
Native or eukaryotic protein expression & purification	Cited protein purification	Same as above plus using a published procedure.	REC052	4-20

Assay Development Modules



Type	Name	Deliverable	Module #	Timeline (wks)
GTP Exchange (fluor. kinetic, 360nm/460nm)	G-protein GTP exchange assay using Mant-GTP	Report with optimized protocol, based on data from titrating four variables ([ionic], [$MgCl_2$], [Mant-GTP] and temp.).	DEV026	4
GTPase assay (abs, endpoint, 650nm)	GTP hydrolysis assay, detecting phosphate	Same as above, except [Mant-GTP] is replaced by [G-protein] and if available [GAP protein].	DEV031	4
Motor ATPase (abs, kinetic, 360nm)	ATP hydrolysis assay, detecting phosphate	Report with optimized protocol, based on data from titrating five variables ([ionic], [$MgCl_2$], [Motor], [microtubules] and temp.).	DEV034	4