EC 70124, a kinase inhibitor of NF-kB pathway, targets initiating cancer cells (cancer stem cells)

Francisco Morís, Managing Director

Bilbao, 21 de septiembre de 2012

Content

1. **The Company**
2. **The Product**
   a) Target Indications
   b) Innovative mechanisms of action
   c) Differential features facing the market
   d) Current status of development
   e) IPR protection
   f) Pitfalls & Risks to be considered
3. **Partnering Opportunities**

Content

1. The Company

Who is EntreChem?

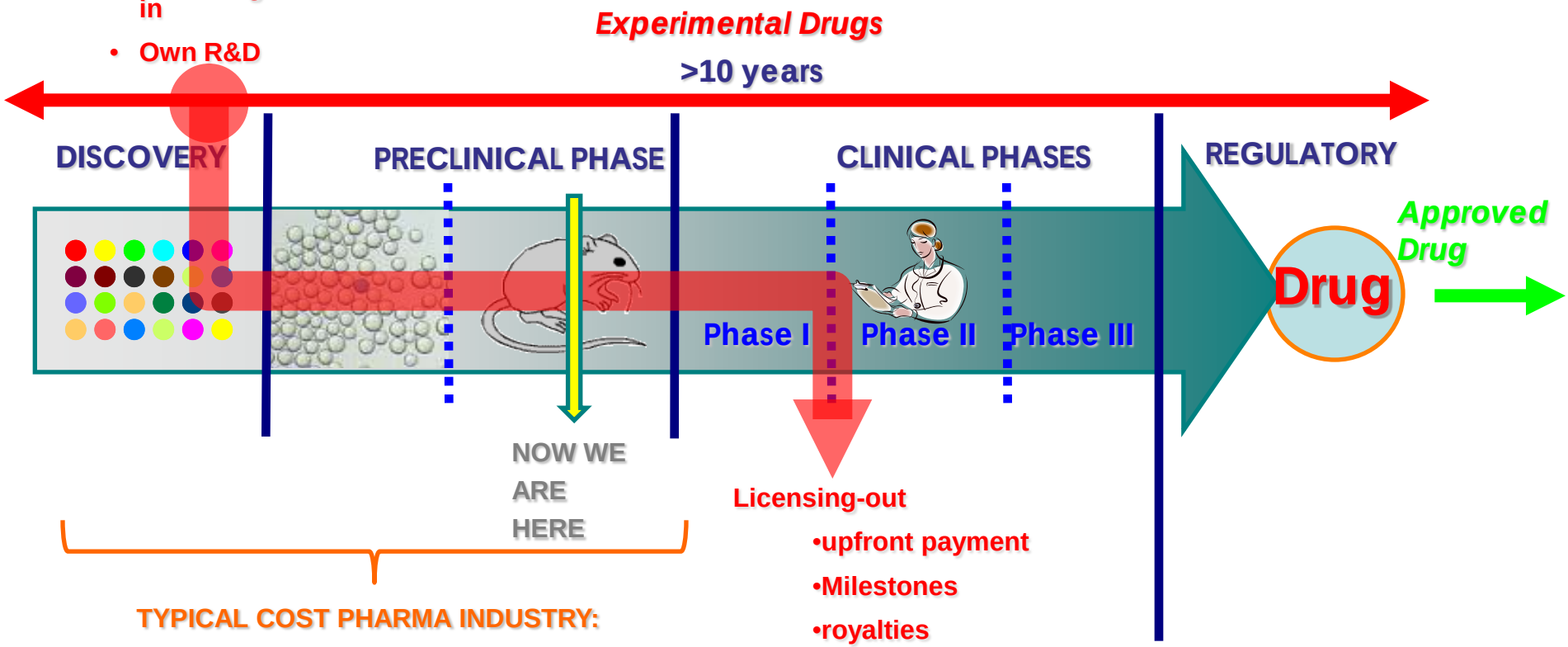
- EntreChem (2006, Oviedo) founded by Prof. José A. Salas Vicente Gotor
  - at the forefront of new developments in Natural Products Drug Discovery
- Seed capital (0.8M€) in 2006, mostly public funds
- Private investors + Public Institutions (1.1M€) in 2010
- Private investors only (1M€) in 2012

EntreChem Mission
EXTRACT VALUE FROM THE SCIENTIFIC RESEARCH OF ITS ACADEMIC COFOUNDERS

- EntreChem has two products in regulatory preclinical for oncology:
  - a transcription modulator (EC-8042)
  - a kinase inhibitor with unique selectivity profile (EC-70124)
- In 18 months, EntreChem could be in the clinic with 2 drug candidates

EntreChem in the Value Chain

- Licensing-in
- Own R&D

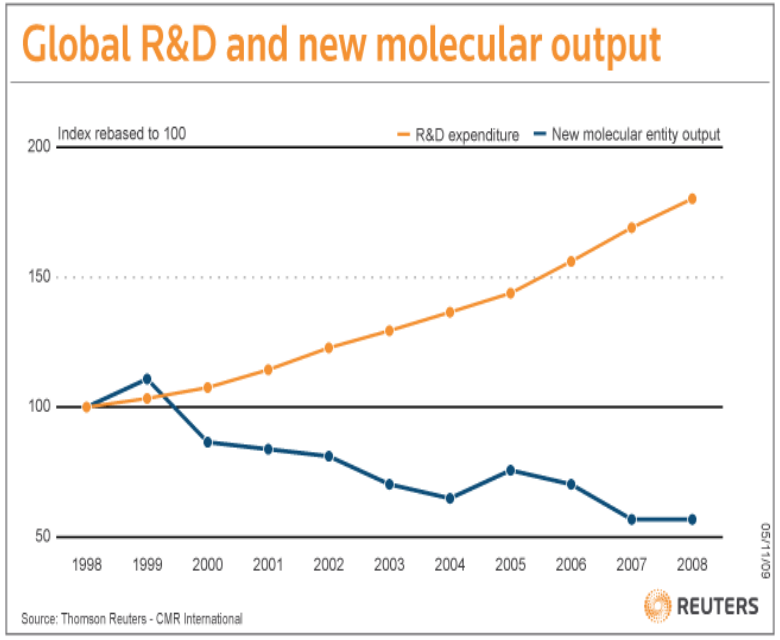


TYPICAL COST PHARMA INDUSTRY:

8 X $250K = $2M annual

ENTRECHEM MAKES MORE WITH LESS

Need for New Molecules



Reuters graphic/Scott Barber

- **Pharma Cos need innovation**
  - internal pipelines becoming non-productive…
  - … but R&D expenses keep growing!
- **Sources of molecules**
  - **Natural products** by fermentation
  - After decades exploiting this space as the source of new medicines, Big Pharma abandons ***natural products R&D*** in the 1990´s
- **Advances in genetic engineering**
  - expand chemical space of ***natural products***
  - actors: academic groups + spin-offs

¡GREAT OPPORTUNITY!

Product Pipeline

EntreChem´s technology enables development of a product pipeline

	Genetic tractability	Diversity Generation / *in vitro* assays	*in vivo* Proof of concept	Formal Preclinical Devlpmt.
Cancer				
Transcription modulator *EC-8042*	→	→	→	**Mithramycin**
multi-kinase inhibitor *EC-70124*	→	→	→	**Staurosporine**
target to be determined	→	→	**novel natural compound**	
other programs				
Neuroprotectant	→	→	**Collismycin**	

Mithralog EC-8042: efficacy

- **EC-8042 scores 66 out of a theoretical maximum of 84**
  - **(42 experiments, QDx4 via i.p. and s.c., 2 points for each condition where >50% reduction)**
- **One of the highest scores ever recorded in the DTP program**
- **MTM A scores 58, since EC-8042 dose is one order of magnitude higher**
  - **potential for therapeutic window exists**

Cmpd	GI50	TGI	LC50	MTD i.p. (mg/kg)	HF/CELL KILL
MTM	-7,7	-5,9	-5,2	6,25	58//Y
EC-8042	-7,5	-5,6	-4,5	200	66/Y

EC-8042				MTM			
2	2	2	0	2	2	2	0
2	2	2	0	2	2	2	2
2	-	2	-	2	0	2	0
2	2	2	2	2	2	0	2
2	-	2	-	2	2	2	0
2	2	2	2	2	2	2	2
2	2	2	0	2	0	2	0
2	2	2	0	2	2	0	0
0	2	2	2	0	0	0	0
-	0	-	0	2	0	0	0
2	0	2	0	0	0	0	0
2	2	2	2	2	2	0	2

Núñez et al., J. Med Chem. ***2012****, 5813-25*

Content

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EC-70124 target indications

- Glioblastoma multiforme
- Head & Neck cancer
- Acute Myeloid Leukemia (AML)
- Prostate cancer

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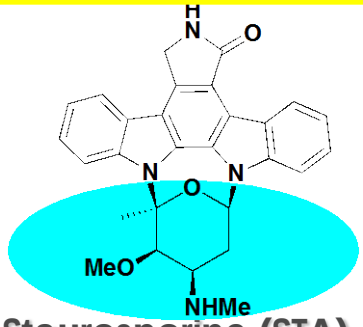
Potent, Selective kinase inhibitors

- Combinatorial biosynthesis allows engineering of the series lead chemical structure in a unique way. Genes from Staurosporine and Rebeccamycin are combined to provide new analogs, especially variations in the sugar moiety (not chemically possible)
- 14 STA-like analogs tested against a panel of 60 kinases.
  - IC50 for combinations kinase-analog showing >70% inhibition at 10nM

- **New potent and selective analogs 100x less cytotoxic than series lead**



Staurosporine (STA)

IC_{50} (nM)	EC70119	EC70120	EC70123	EC70124	EC70127	EC70128
AurA	-	>10	5.0	>10	>10	6.7
AurB	-	-	6.8	-	-	4.5
Chk1	2.4	1.0	-	4.9	>10	-
Dyrk1a	-	4.0	-	-	>100	>10
Ftl3	0.59	0.57	0.54	0.56	-	0.43
FGFR1	-	8.9	>10	-	>100	>10
HGK	-	0.78	-	-	>10	>10
Ikkb	-	0.17	-	<0.03	>100	>10
Jak2	0.43	0.50	0.57	0.74	0.53	1.2
KDR	-	3.7	-	0.55	>10	>10
SYK	-	1.0	-	1.1	>10	2.3

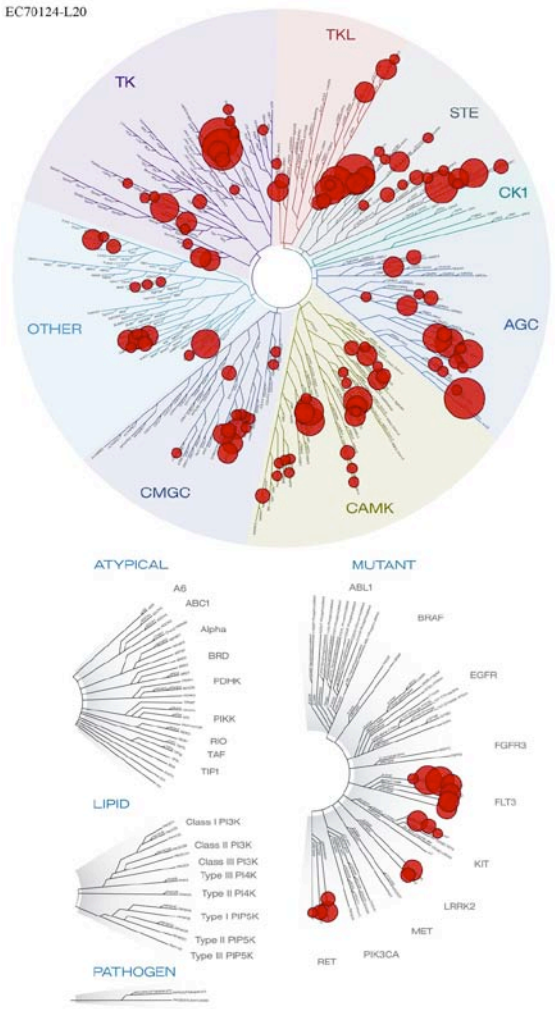
Sánchez et al, Chem. Commun., 2009, 4118–4120

EC-70124 kinase assay reliability?

- **Measured in 3 different platforms (1 affinity, 2 activity)**
  - ✦ **10 kinases tested, IKK-beta results 3 orders of magnitude difference depending on assay technique**

	Caliper (electrophoretic mobility) IC_{50} , nM	Discoverx(Affinity) Kd, nM	Proqinase (radioactivity) IC_{50} , nM
AurA	16	21	64
Chk1	9.9	9.2	35
EGF-R wt	>100	2200	2000
FAK	-	320	1400
FLT3 wt	2.4	5	19
Ikk-alpha	>100	560	450
Ikk-beta	2.1	120	1600
Ikk-epsilon	-	120	79
SYK	2.7	7.8	16
KDR (VEGF R2)	0.94	57	19

EC-70124 affinity report



392 non-mutant kinases assayed for affinity (Ambit platform)

Selectivity Score Type	Number of hits	Selectivity score
S (35)	117	0.298
S (10)	63	0.161
S (1)	16	0.041

63 kinases >90% inhibited at 100nM
16 kinases >99% inhibited at 100nM

EC-70124 cellular data: Carna

Table I. Summary of compound activities versus the indicated ACD cell-based PTK assays.

Assay	% Inhibition 100 nM	IC_{50} (nM) Axitinib	Saracatinib	Sorafenib	SYK Inhibitor	Pazopanib	Tofacitinib
ACD1000	18.7	2,115	> 3,160	> 3,160	> 3,160	> 3,160	1,104
ALK	27.5						
AXL	10.9						
EphB1	34.1		341				
FGFR1	66.3	365					
FLT1	83.0			345			
FLT3	89.7			15.8			
FLT4	98.0			127			
INSR	66.4						
JAK1	55.4						
JAK2	43.0						
JAK3	18.7						
KDR	81.3			31.9			
KIT	16.4			13.2			
PDGFRb	30.4					62.2	
PTK7	50.7						
RET	72.8			360			
ROR1	87.6						
RYK	68.8						
SYK	98.8				816		
TRKA	92.5				58.3		
TRKB	65.7				224		
TRKC	86.9				110		
TYK2	21.8						
ZAP70	42.2						294

EC-70124 cellular data: Proqinase

Table 5: IC_{50} values of indicated compounds for inhibition of Aurora-B, ALK and AXL cellular kinase activity. Each compound was tested in 8 concentrations (in duplicate).

#	compound name	unit	IC50			
			ALK	Aurora-B	AXL	FLT3-wt
C1	EC-70124-L20	M	2,6E-06	2,7E-07	2,3E-07	3,1E-08
R1	Crizotinib	M	3,2E-07	-	-	-
R2	Staurosporine	M	-	1,8E-08	-	-
R3	Sunitinib	M	-	-	1,3E-06	2,0E-08

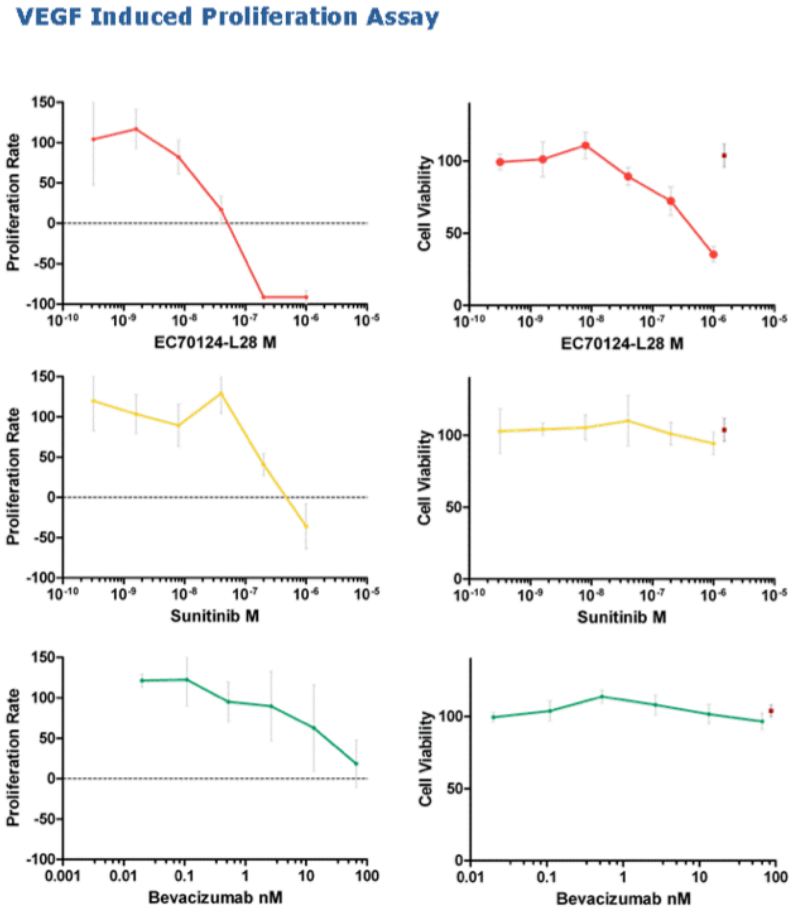
#	compound name	unit	IC50			
			FLT3-DY	FLT3-ITD	KIT	PDGFR-β
C1	EC-70124-L20	M	4,1E-09	3,2E-08	6,9E-08	2,5E-08
R3	Sunitinib	M	9,1E-08	6,0E-08	3,1E-10	3,1E-09

#	compound name	unit	IC50		
			S6K	VEGFR-2	VEGFR-3
C1	EC-70124-L20	M	3,5E-08	1,6E-08	5,0E-09
R3	Sunitinib	M	-	1,1E-09	2,2E-09
R4	Everolimus	M	6,1E-11	-	-

Color Code:

IC50	IC50 (M) > 1E-05
IC50	IC50 (M) ≤ 1E-05 and > 1E-07
IC50	IC50 (M) ≤ 1E-07 and > 1E-08
IC50	IC50 (M) ≤ 1E-08

EC-70124 not anti-angiogenic



- KDR activity in biochemical assays not correlated to typical anti-angiogenic activity
- VEGF induced HUVEC proliferation assay correlates well with lack of cell viability, not with typical anti-angiogenic effect (as in sunitinib or bevacizumab)

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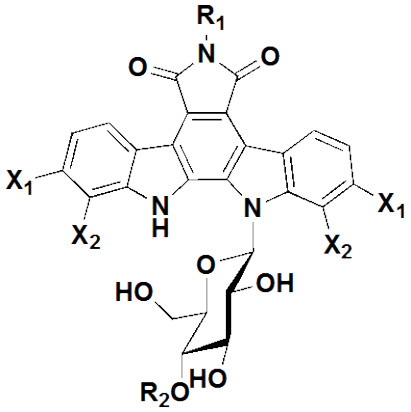
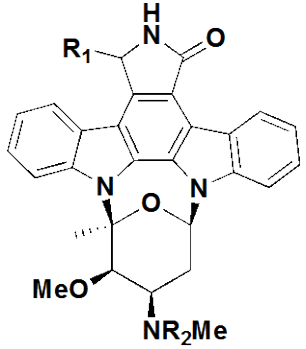
3.

Indolocarbazoles in Clinical Trials

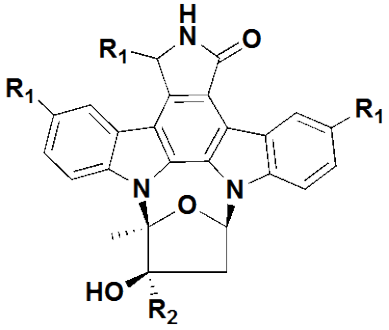
- > 1/3 of R&D in pharma now spent on kinase inhibitors
- historical track record of safety in clinical trials
- Midostaurin now in Phase III



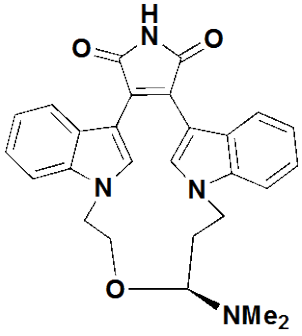
Becatecarin (cancer, Helsinn)
Edotecarin (cancer, Banyu/Pfizer)



UCN-01 (cancer, Kyowa Hakko Kogyo)
Midostaurin (cancer, Novartis)



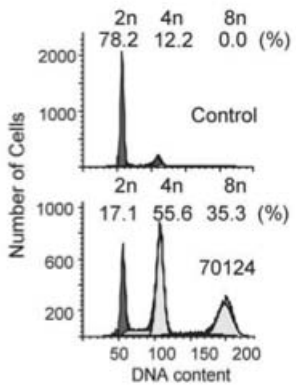
CEP-1347 (parkinson, Cephalon)
CEP-701 (cancer, Cephalon)



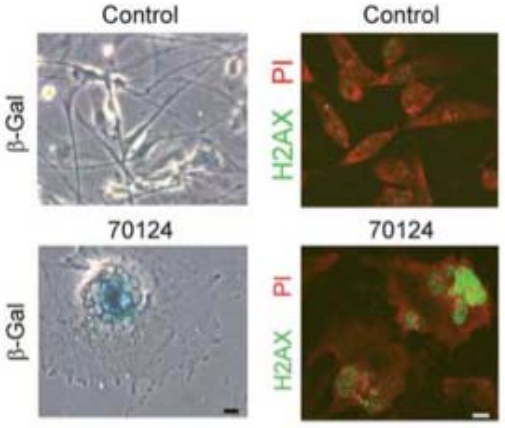
Ruboxistaurin (Diabetic Retinopathy, Lilly)

EC-70124 for cancer: Glioblastoma differentiation therapy

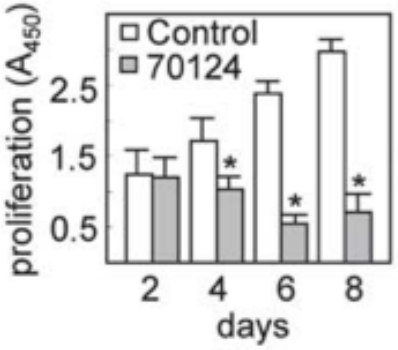
- **NF-kB is activated in Glioblastoma Initiating Cells (GICs) undergoing differentiation**
  - **Inhibiton of NF-kB promotes growth arrest, differentiation and senescence**
- **EC-70124 drives differentiating GICs into senescence**
  - **Tested on GICs derived from surgical specimens**



polyploidy



senescence *DNA double-strand breaks*

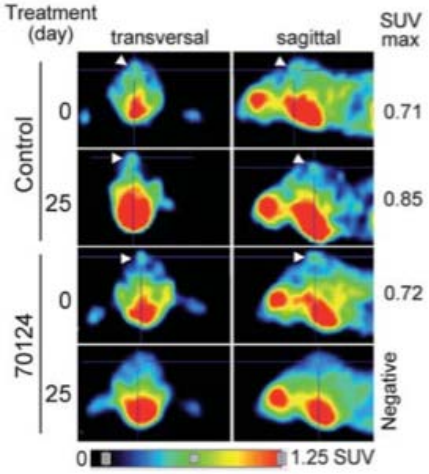


reduced proliferation capacity

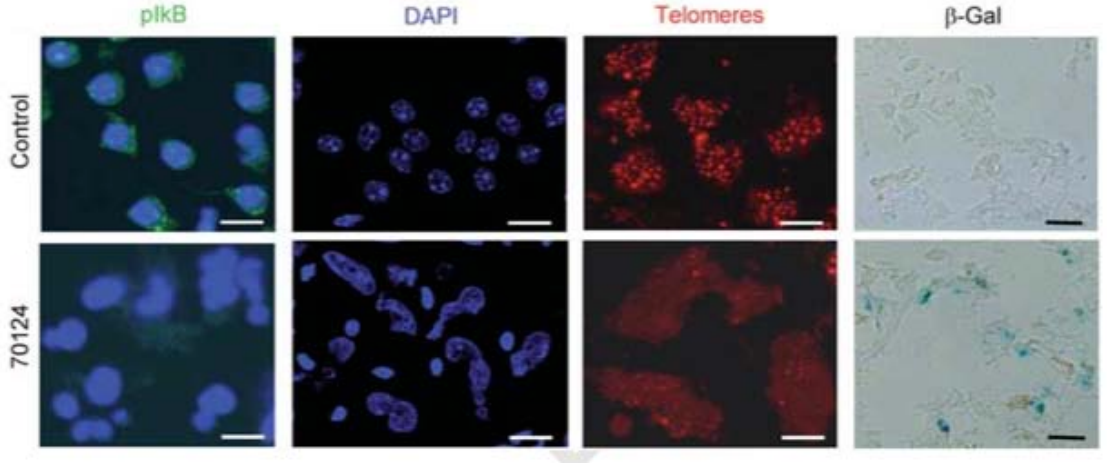
Nogueira et al, Oncogene. **2011** *30(32)*:3537-48

EC-70124 for cancer: Glioblastoma differentiation therapy

- NF-kB is activated in GBInitiating Cells (GICs) undergoing differentiation
  - Inhibiton of NF-kB promotes growth arrest, differentiation and senescence
- EC-70124 drives differentiating GICs into senescence
  - Tested on GICs derived from surgical specimens
- Orthotopic xenograft model (brain): mice bearing GIC-derived tumors
  - Squash preparations of tumor tissues match observations of *in vitro* experiments



µ-PET data
Nogueira et al, Oncogene. **2011** *30(32)*:3537-48



loss of NF-kB activity *aberrant nuclei* *telomere shortening* *senescence*

EC-70124 anticipated efficacy data:

- AML represents the highest medical need in leukemia:
  - EC-70124 targets kinases involved in AML: Fllt3, VEGF2, SYK.
  - IC50 in nM range in certain leukemia and myeloproliferative disorders
  - Human ex-vivo data (peripheral blood and bone marrow) : inhibition at 0.5-1uM

- Prostate cancer
  - Very active in prostate cancer stem cells (prostatospheres)
    - Selective effect on stem population
  - Mechanism of action linked to NF-kB pathway inhibition

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2. **The Product**
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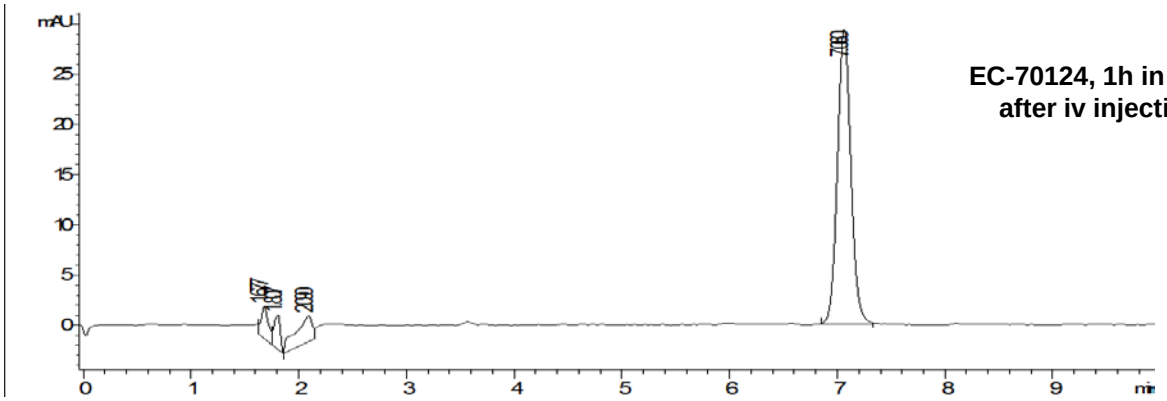
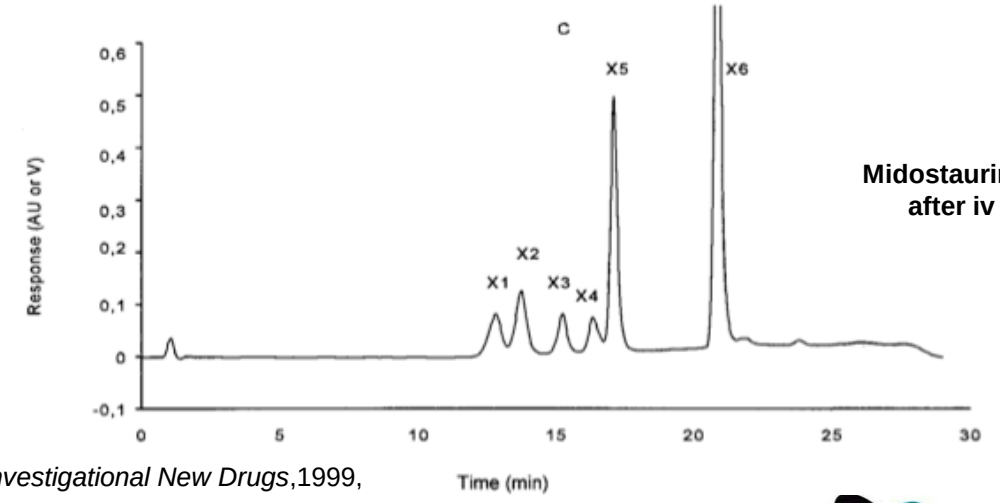
EC-70124 development summary:

- hERG channel assay
- CYP inhibition profile
- Microsome stability in 3 species
- MTD in mice in single, multiple dose schedules, iv, po, ip routes
- MTD in rat iv route
- PK in mice
- PPB functional assay
- Bioanalytical validation
- GLP 2 weeks study in rats and dogs (ongoing)

EC-70124 metabolic stability



EC-70124, 1h in mice after iv injection

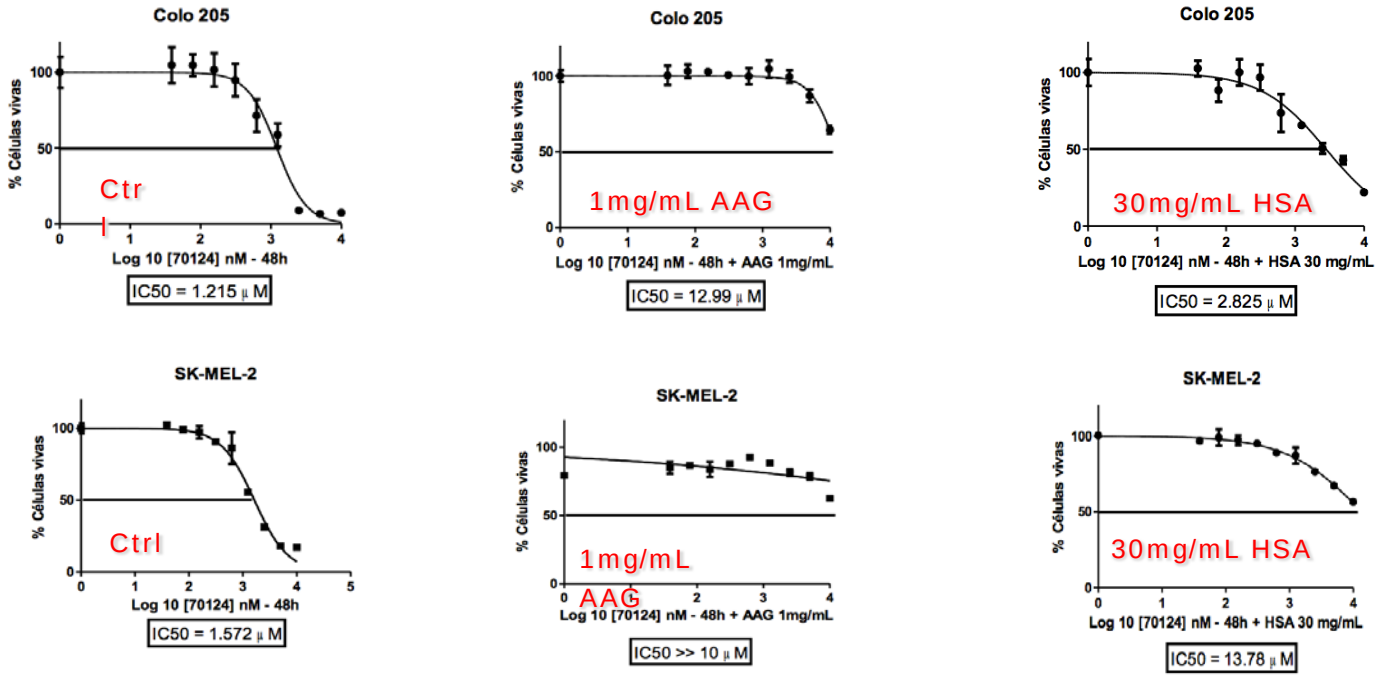


Midostaurin, 1h in mice after iv injection

van Gijn *et al, Investigational New Drugs*,1999, 17, 29-41

EC-70124 plasma binding

- **HSA and AAG two of the main human proteins**



- **IC_{50} one order of magnitude higher**
  - **Compared to 2 orders of magnitude for midostaurin Fabro *et al. Pharmacol. Ther*. 1999 (82), 293–301)**

Content

1.
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   a)
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   f)
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Intellectual Property

PROGRAM	Application/ Publication	Licensing/ property
Kinase inhibitors *EC-70124*	*ES P200801077* WO2009125042 US2011136753 EP2277885	Codeveloped and licensend-in
Kinase inhbitors	ES P200102312 WO2003033706 US2008004326 EP1443113	Licensed-in

- New substance Patents
  - Highest degree of protection
  - Expire in 2028
- Potential for new IP development
  - Use, combinations, formulation patents to extend protection beyond 2030

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EC-70124 pitfals and risks:

- Focus on intravenous administration:
  - Could be seen as not attractive…
  - … but not such an issue for oncology applications (improves compliance)
  - Applications outside oncology (inflammation) may requiere p.o.

- Risks:
  - No short terms major risks identified
  - Longer term: efficacy in humans (Phase II) as indolocarbazole´s track record suggests

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EC-70124 partnering opportunities:

- In oncology for intravenous administration
- Develop an oral formulation for other applications
- Partner with or without experience in oncology