Overcoming TRK inhibitors resistance: pre-clinical and clinical evidences

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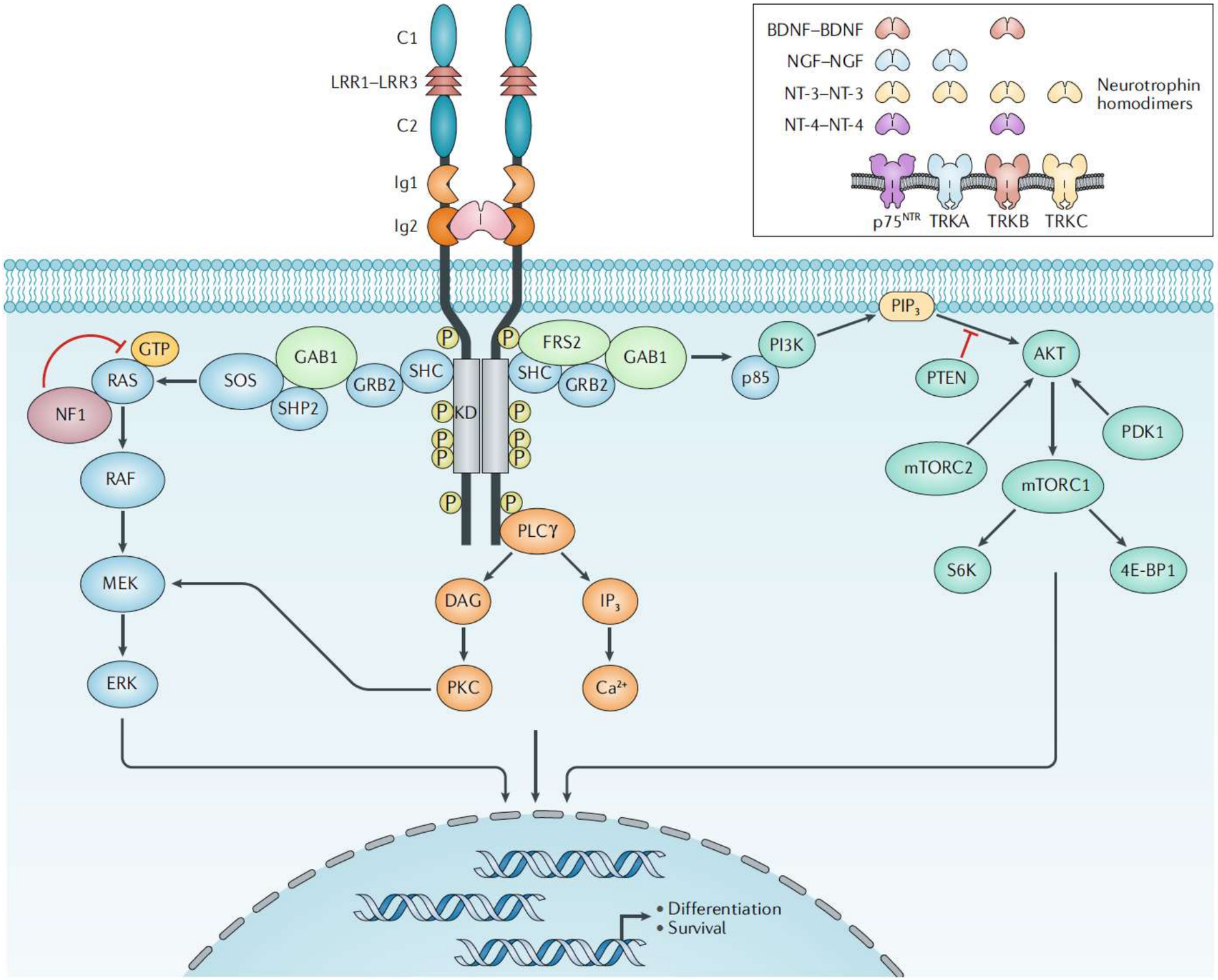
V GETHI symposium, Madrid 19-11-2019

Tropomyosin receptor kinases (Trks)



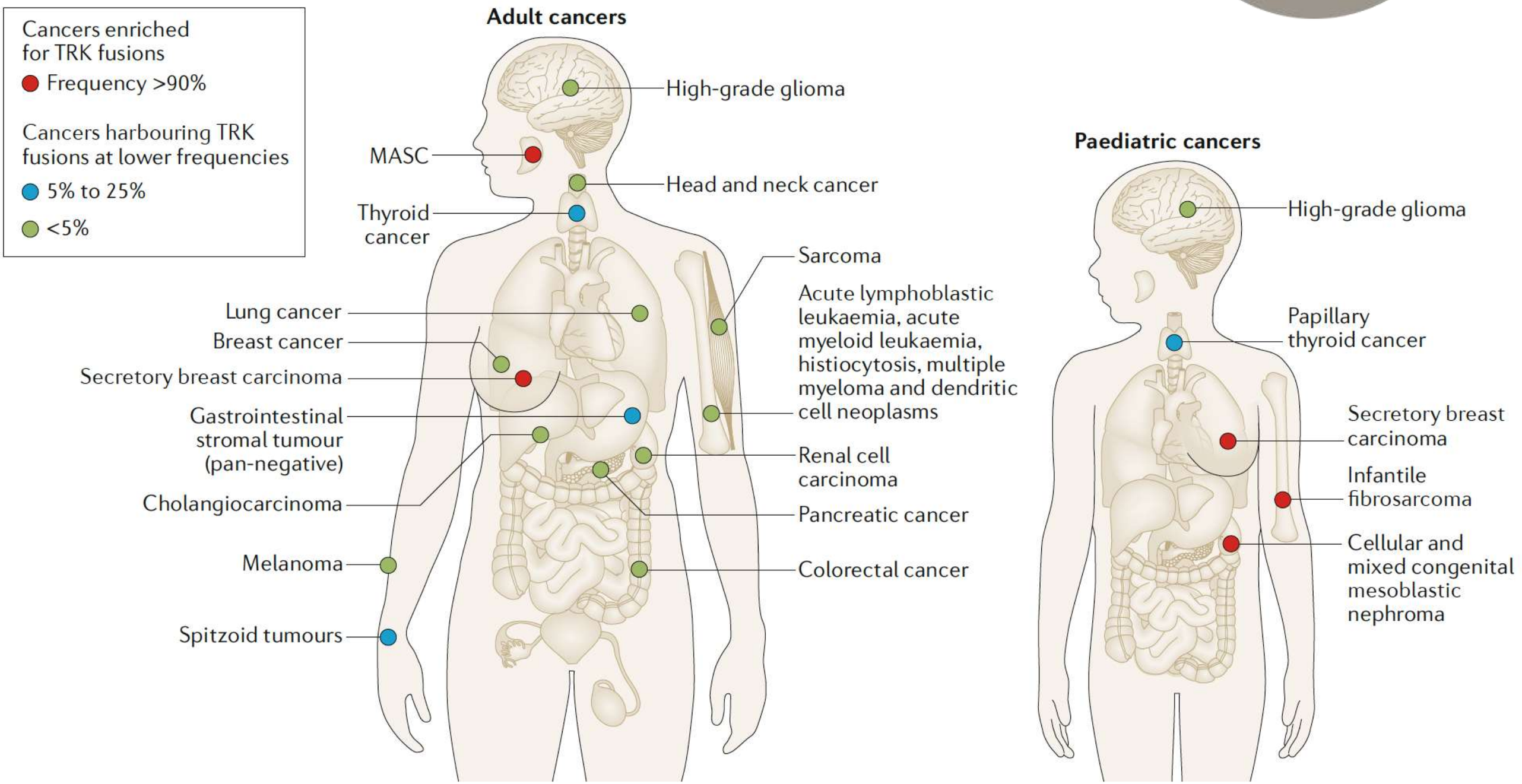
TrkA (NTRK1) :
Pain, thermoregulation

TrkB (NTRK2) :
Movement, memory, mood, appetite, body weight

TrkC (NTRK3) :
Proprioception

Cocco E. et al. (2018)

NTRK fusions across multiple tumor types



An estimated 1500 – 5000 harbour TRK fusion positive cancers in United States annually

Cocco E et al. (2018)

TRK inhibition:
a tumor-agnostic treatment strategy

Several TKIs with varying degrees of activity against TRKA, TRKB and/or TRKC are available, which can broadly be grouped into: i) multi- kinase inhibitors with activity against a range of targets including TRK or ii) more selective TRK inhibitors

1st generation:

i) **Entrectinib** (Rozlytrek), crizotinib, cabozantinib, lestaurtinib, altiratinib, foretinib, ponatinib, nintedanib, merestinib, MGCD516, PLX7486, DS-6051b and TSR-011.

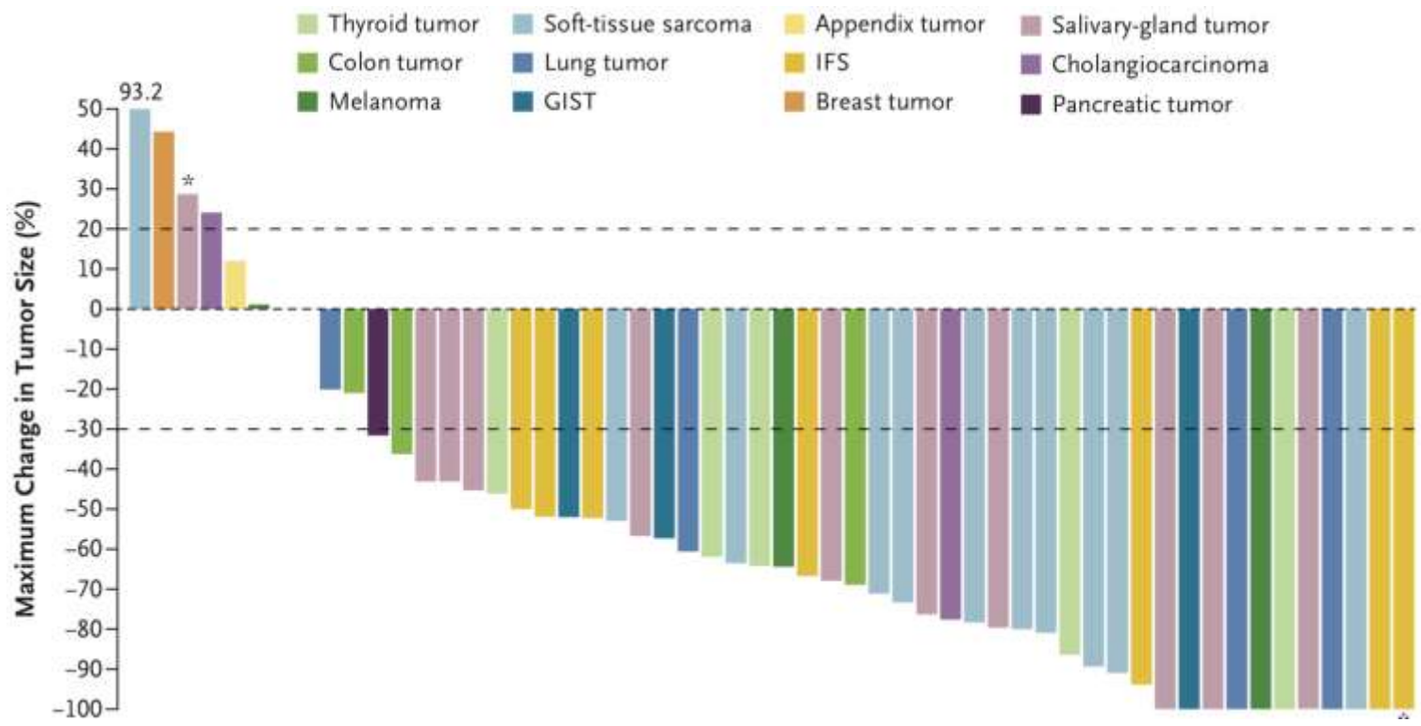
ii) **Larotrectinib** (Vitrakvi, LOXO-101)

2nd generation:

LOXO-195

Efficacy of Larotrectinib in *TRK* Fusion–Positive Cancers in Adults and Children

Alexander Drilon, M.D., Theodore W. Laetsch, M.D., Shivaani Kummar, M.D., Steven G. DuBois, M.D., Ulrik N. Lassen, M.D., Ph.D., George D. Demetri, M.D., Michael Nathenson, M.D., Robert C. Doebele, M.D., Ph.D., Anna F. Farago, M.D., Ph.D., Alberto S. Pappo, M.D., Brian Turpin, D.O., Afshin Dowlati, M.D., et al.

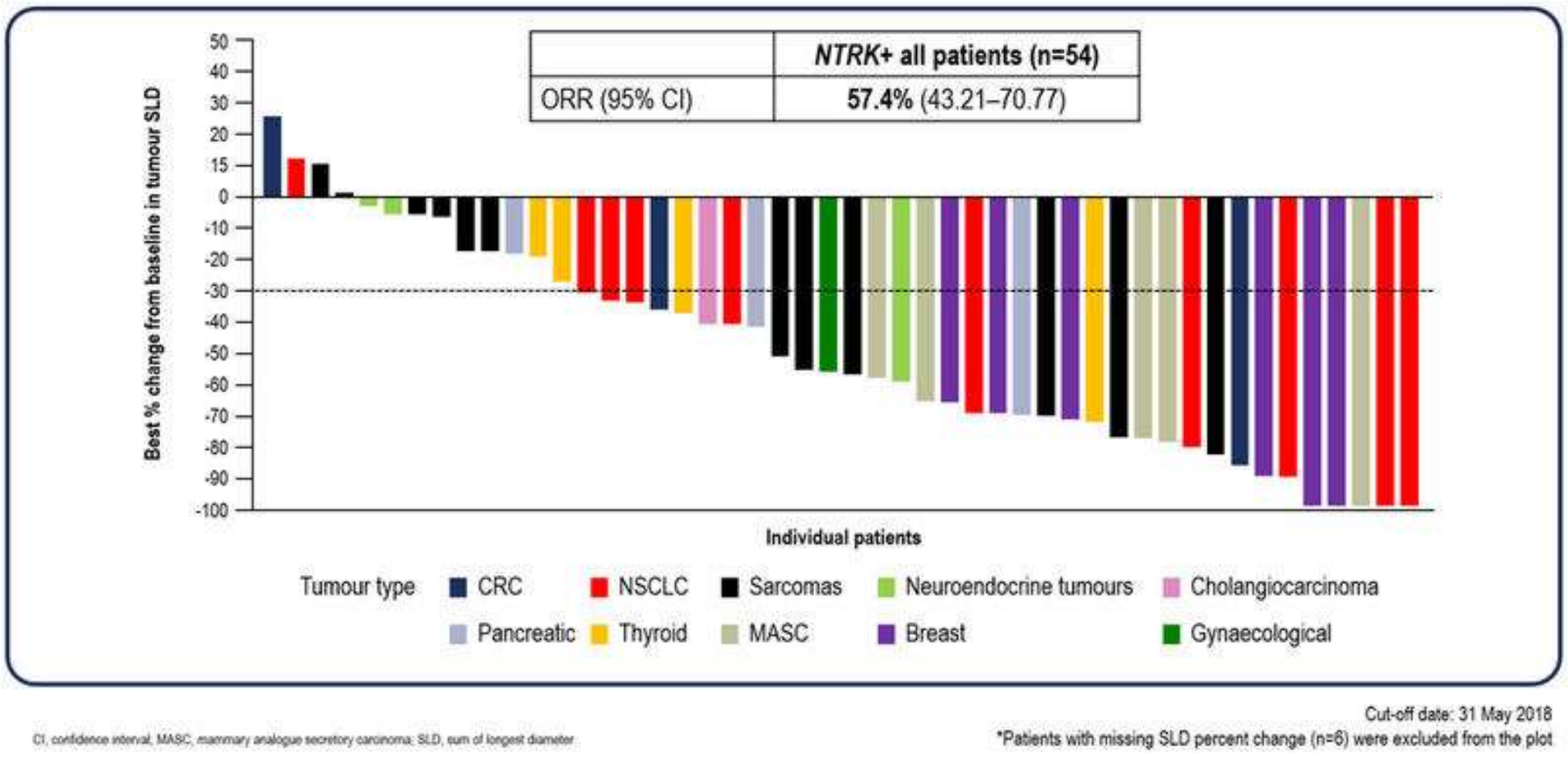


ORR

75%(CI 61%-85%)

Drilon et al., N Engl J Med (2018)

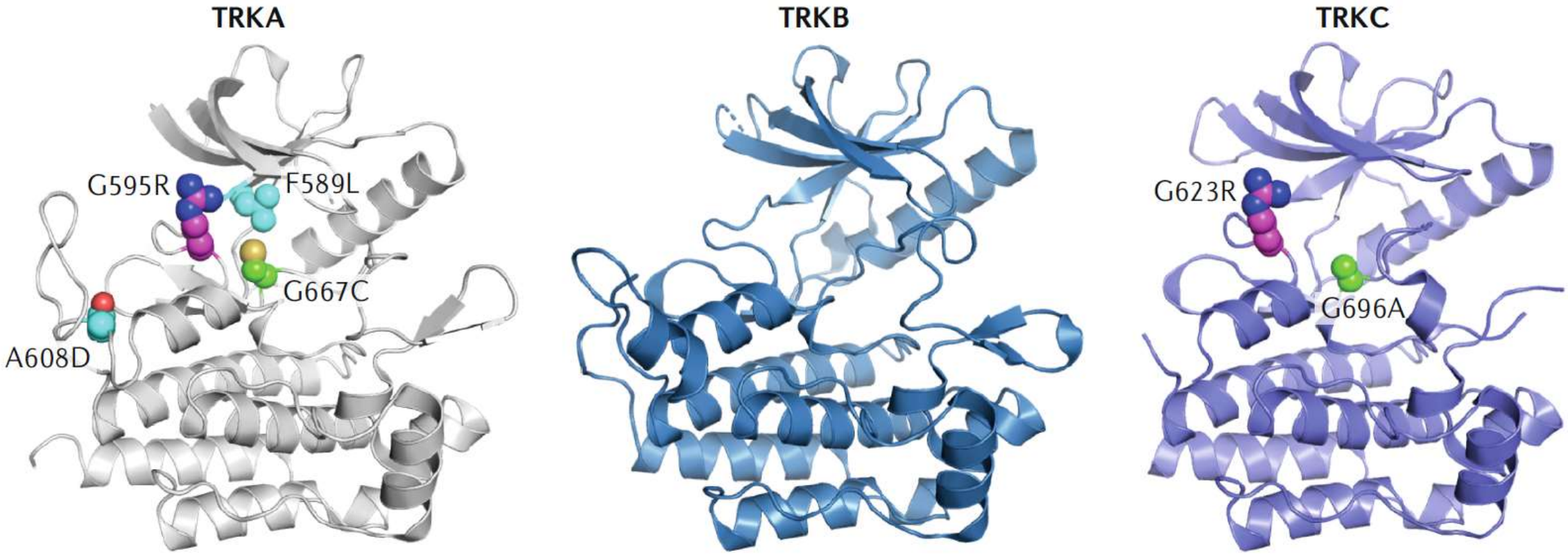
Clinical efficacy of Entrectinib



ESMO (2018)

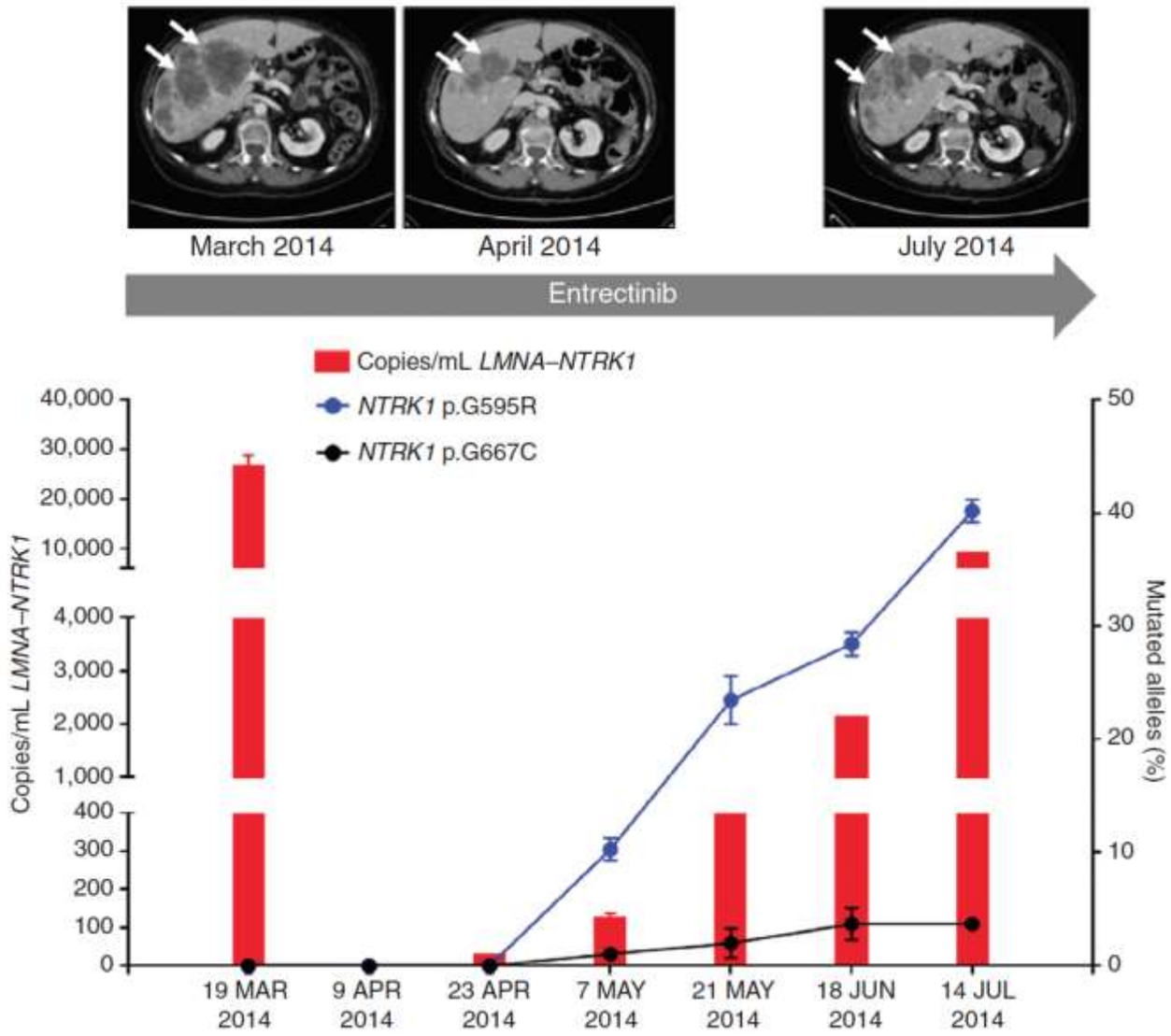
On August 15, 2019, the oral TRK, ROS1, and ALK kinase inhibitor entrectinib was granted accelerated approval for treatment of adult patients and pediatric patients 12 years of age or older with solid tumors that have a neurotrophic tyrosine receptor kinase (*NTRK)*-gene fusion without a known acquired resistance mutation, are metastatic or if surgical resection is likely to result in severe morbidity, and have experienced disease progression following treatment or have no satisfactory standard therapy. Entrectinib also was approved for the treatment of adults with *ROS1*-positive metastatic non–small cell lung cancer (NSCLC).

TRK inhibitors resistance: solvent front mutations



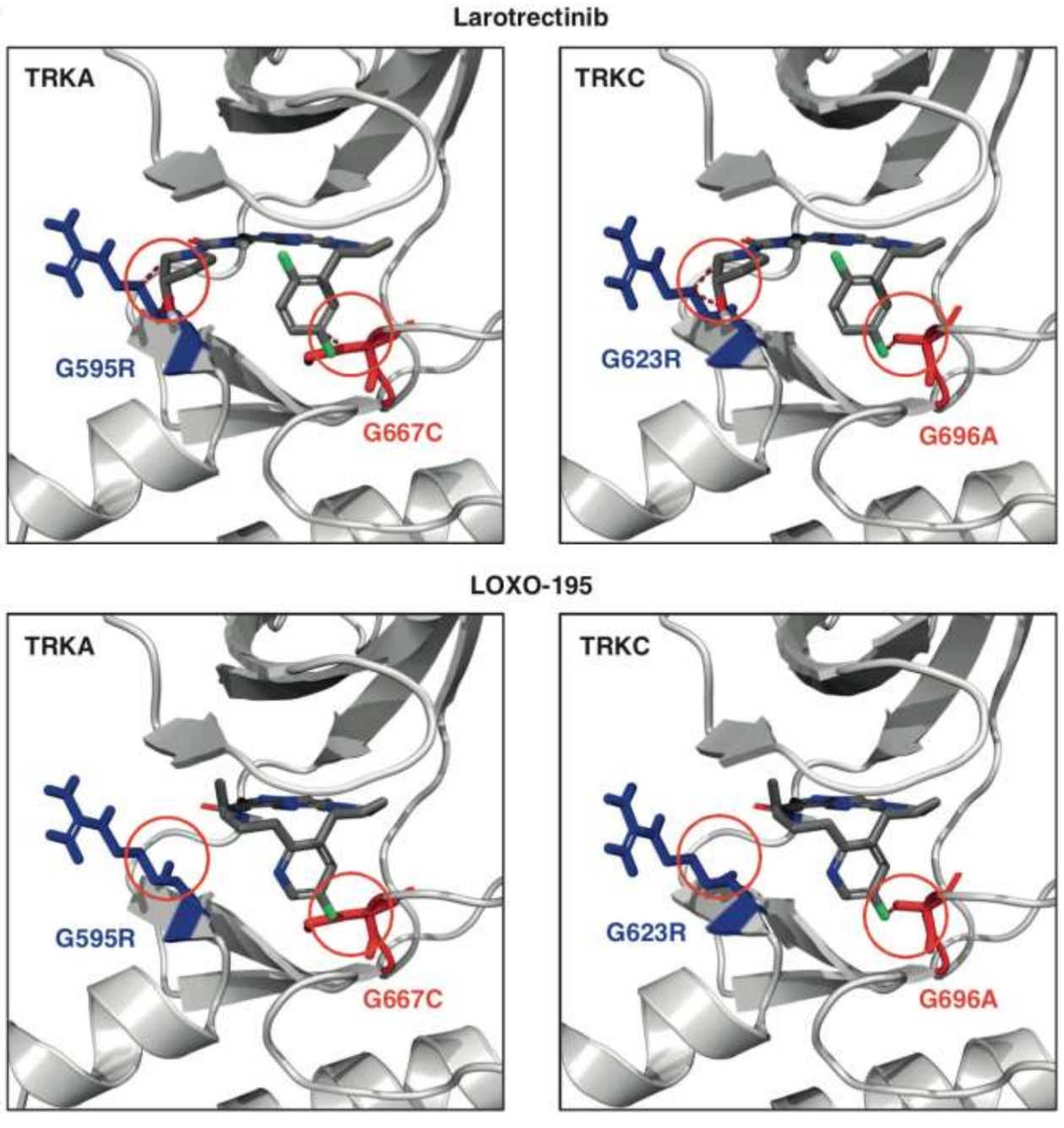
Cocco E et al. (2018)

TRK inhibitors resistance: early clinical evidences



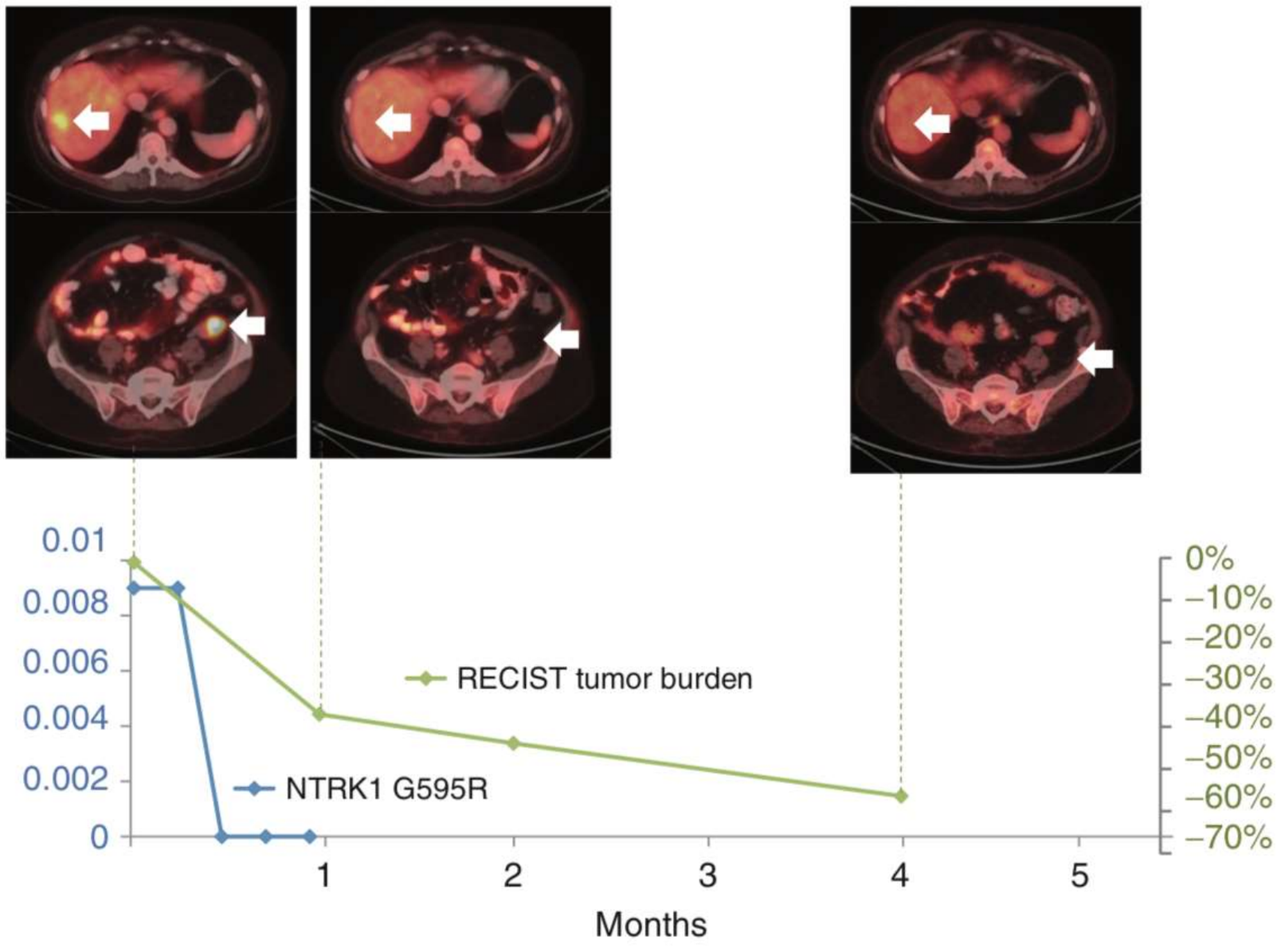
Russo A. et al., Cancer Discovery (2016)

LOXO-195: 2nd generation TRKi



Drillon et al. (2017)

LOXO-195 early clinical experience



Drillon et al. (2017)

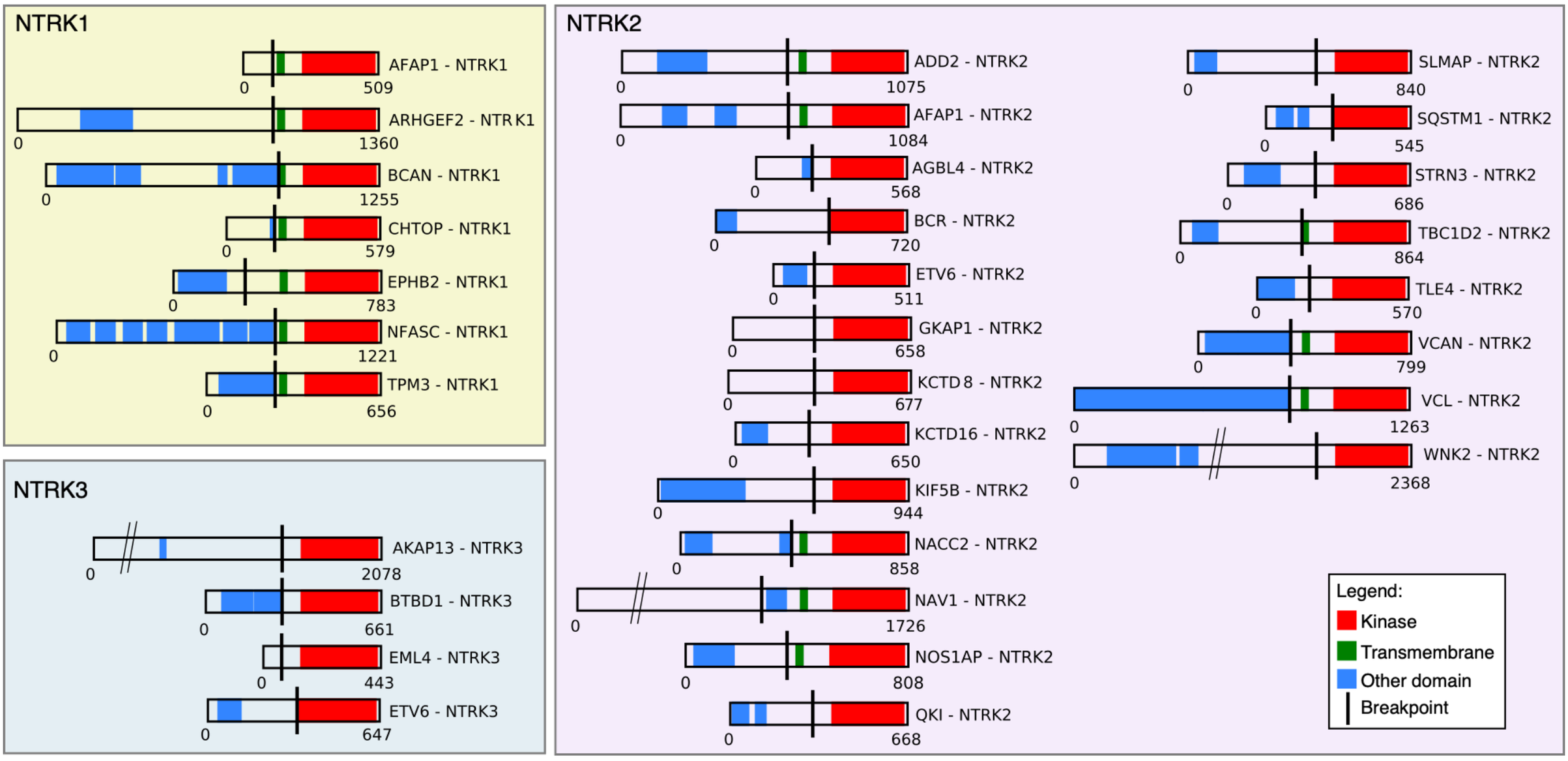
*TRK*ing down novel therapeutic targets in gliomas

NTRK fusion in gliomas

NTRK1		**1q23.1**	**neurotrophic receptor tyrosine kinase 1**	
AFAP1_NTRK1	AFAP1	4p16.1	actin filament associated protein 1	GBM
ARHGEF2_NTRK1	ARHGEF2	1q22	Rho/Rac guanine nucleotide exchange factor 2	GBM
BCAN_NTRK1	BCAN	1q23.1	brevican	GBM, Low-grade glioneuronal tumor
CHTOP_NTRK1	CHTOP	1q21.3	chromatin target of PRMT1	GBM
EPHB2_NTRK1	EPHB2	1p36.12	EPH receptor B2	LGG
NFASC_NTRK1	NFASC	1q32.1	neurofascin	GBM
TPM3_NTRK1	TPM3	1q21.2	tropomyosin 3	NBS-HGG (infant)
NTRK2		**9q21.33**	**neurotrophic receptor tyrosine kinase 2**	
ADD2_NTRK2	ADD2	2p13.3	adducin 2	HGG (infant)
AFAP1_NTRK2	AFAP1	4p16.1	actin filament associated protein 1	LGG
AGBL4_NTRK2	AGBL4	1p33	ATP/GTP binding protein-like 4	NBS-HGG (infant)
BCR_NTRK2	BCR	22q11.23	BCR, RhoGEF and GTPase activating protein	GBM
ETV6_NTRK2	ETV6	12p13.2	ETS variant 6	PXA (infant)
GKAP1_NTRK2	GKAP1	9q21.32	G kinase anchoring protein 1	GBM
KCTD8_NTRK2	KCTD8	4p13	potassium channel tetramerization domain containing 8	GBM
KCTD16_NTRK2	KCTD16	5q31.3	potassium channel tetramerization domain containing 16	HGG (infant)
KIF5B_NTRK2	KIF5B	10p11.22	kinesin family member 5B	HGG (infant)
NACC2_NTRK2	NACC2	9q34.3	NACC family member 2	Pilocytic astrocytoma
NAV1_NTRK2	NAV1	1q32.1	neuron navigator 1	LGG (pediatric)
NOS1AP_NTRK2	NOS1AP	1q23.3	nitric oxide synthase 1 adaptor protein	Astrocytoma
QK_NTRK2	QKI	6q26	QKI, KH domain containing, RNA binding	Pilocytic astrocytoma
SLMAP_NTRK2	SLMAP	3p14.3	sarcolemma associated protein	Ganglioglioma (pediatric)
SQSTM1_NTRK2	SQSTM1	5q35.3	sequestosome 1	LGG
STRN3_NTRK2	STRN3	14q12	striatin 3	Ganglioglioma
TBC1D2_NTRK2	TBC1D2	9q22.33	TBC1 domain family member 2	GBM
TLE4_NTRK2	TLE4	9q21.31	transducin like enhancer of split 4	Ganglioglioma
VCAN_NTRK2	VCAN	5q14.2	versican	LGG
VCL_NTRK2	VCL	10q22.2	vinculin	NBS-HGG infant
WNK2_NTRK2	WNK2	9q22.31	WNK lysine deficient protein kinase 2	Complex glioneuronal tumor
NTRK3		**15q25.3**	**neurotrophic receptor tyrosine kinase 3**	
AKAP13_NTRK3	AKAP13	15q25.3	A-kinase anchoring protein 13	LGG
BTBD1_NTRK3	BTBD1	15q25.2	BTB domain containing 1	NBS-HGG (infant)
EML4_NTRK3	EML4	2p21	echinoderm microtubule associated protein like 4	GBM
ETV6_NTRK3	ETV6	12p13.2	ETS variant 6	NBS-HGG (infant), LGG (pediatric)
ZNF710_NTRK3	ZNF710	15q26.1	zinc finger protein 710	GBM

Oldrini B et al., unpublished

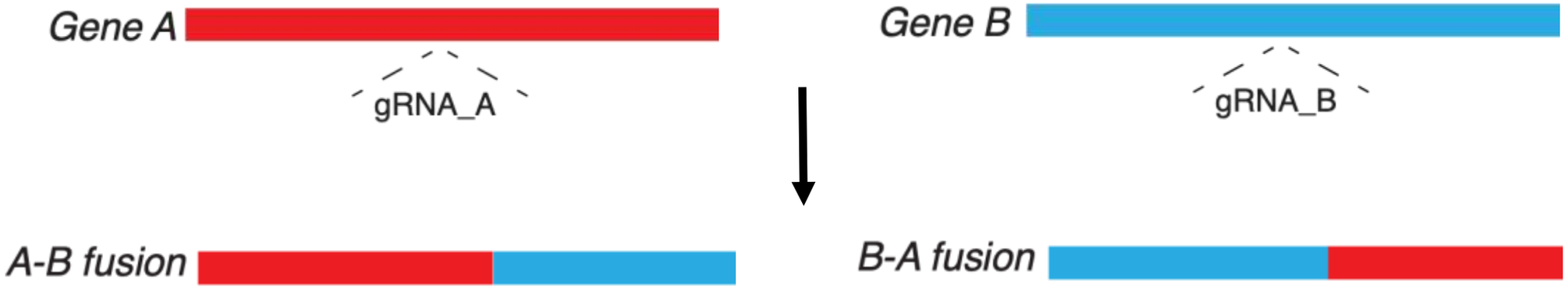
NTRK fusions in gliomas maintain an intact kinase domain



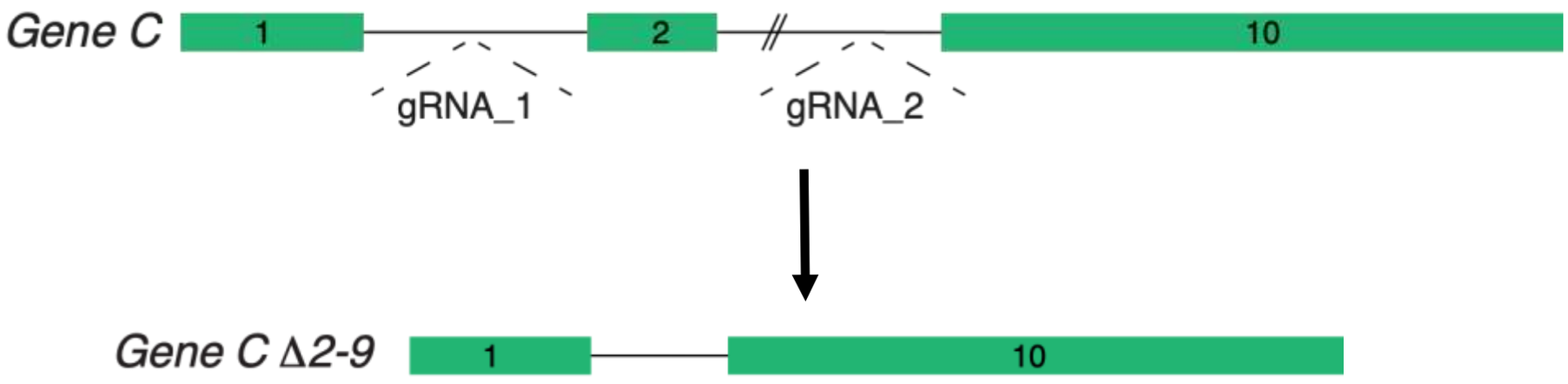
Oldrini B et al., unpublished

CRISPR/Cas9 mediated genomic rearrangements

- Gene fusions

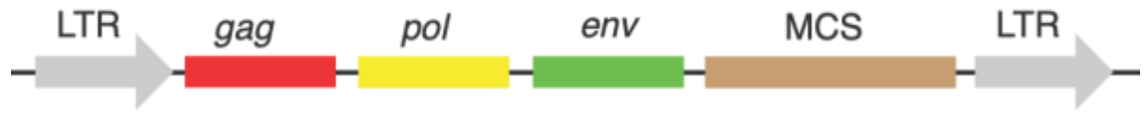


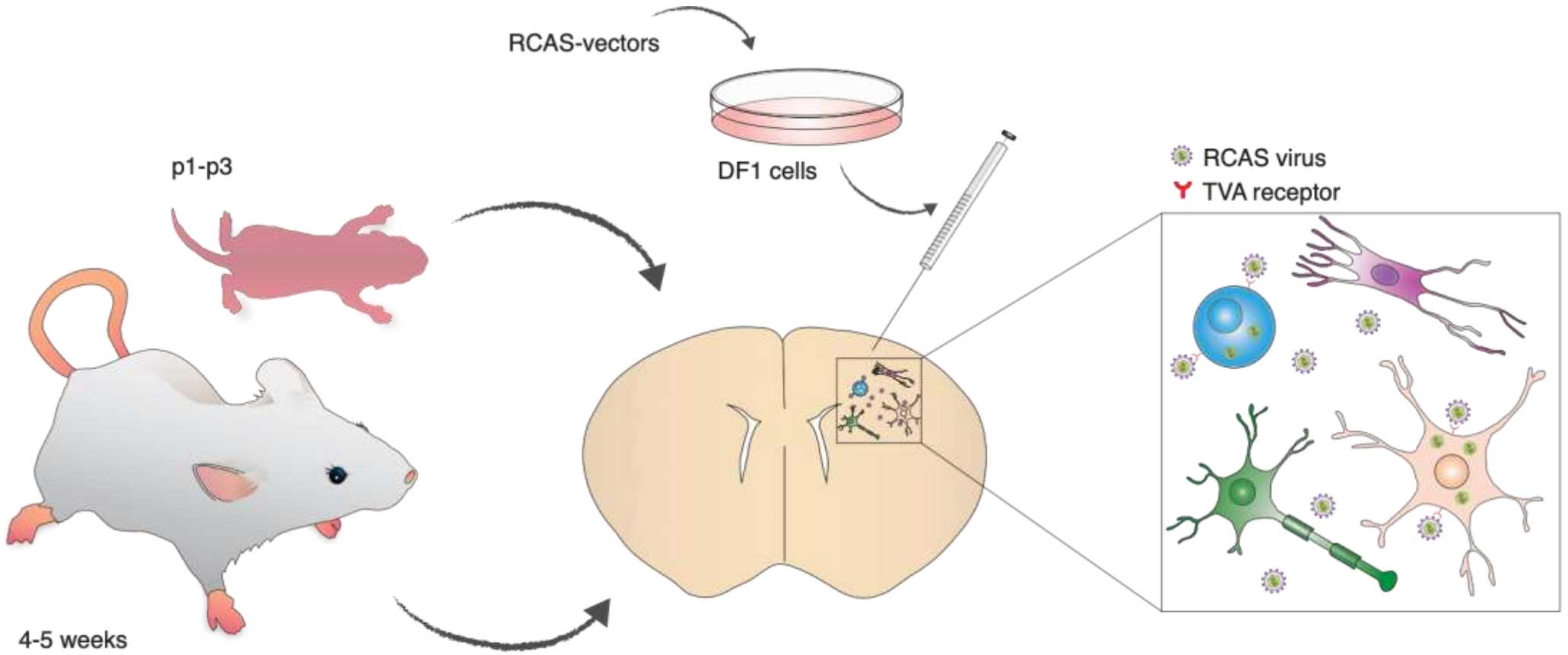
- Deletions

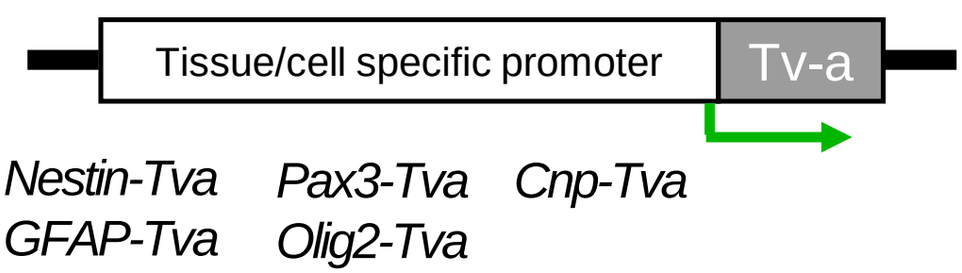


RCAS-Tva system

RCAS: Replication-competent avian sarcoma-leukosis virus long terminal repeat with splice acceptor







Nestin-Tva *Pax3-Tva* *Cnp-Tva*
GFAP-Tva *Olig2-Tva*

RCAS/Tva-CRISPR/Cas9 model

Nestin-Tva x *Rosa26 LSL-Cas9-GFP* x *Nestin-Cre*

GFAP-Tva *GFAP-Cre*

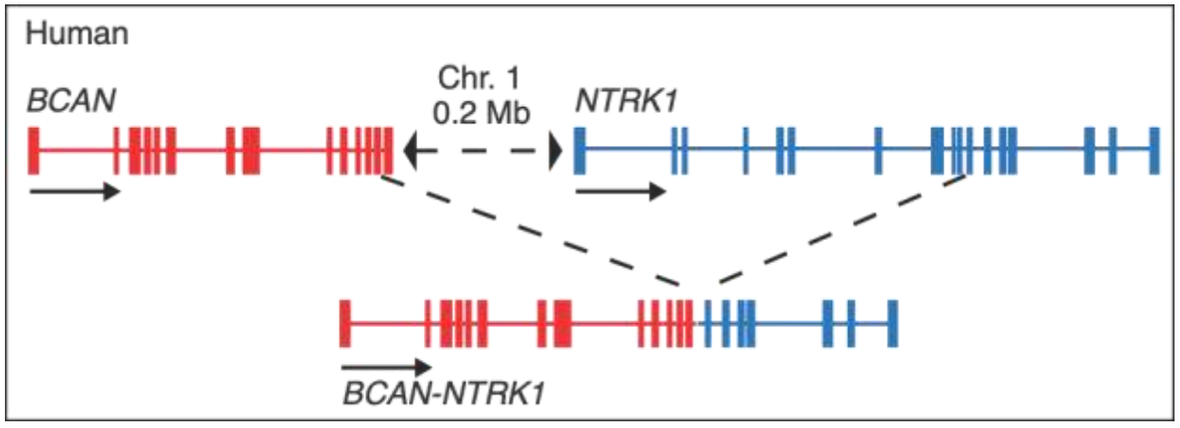
Rosa26 LSL-Tva *“Your fav.”-Cre*

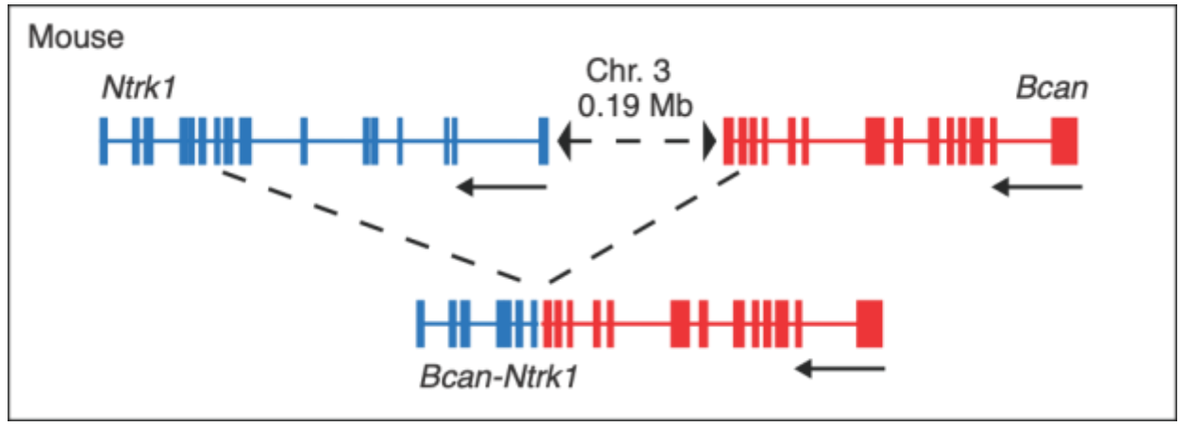
Tissue specific Tva+; Cas9+ mouse strains

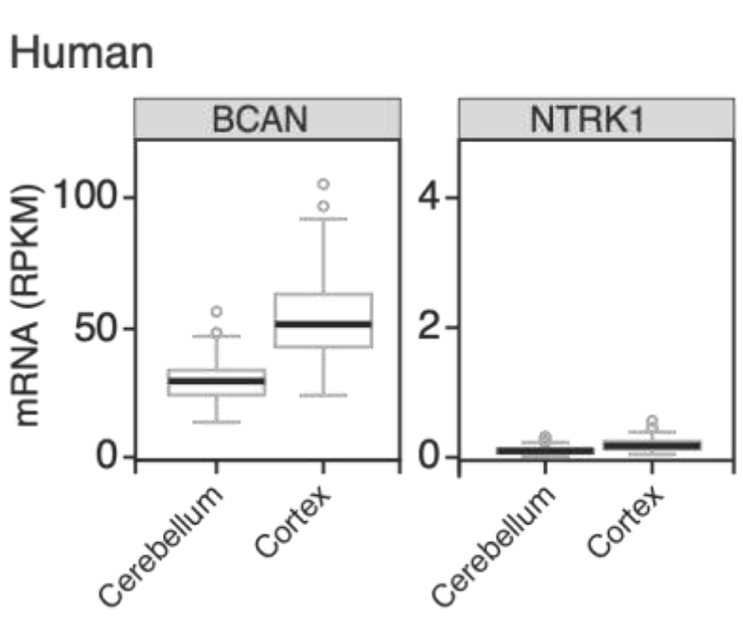
Somatic genome editing

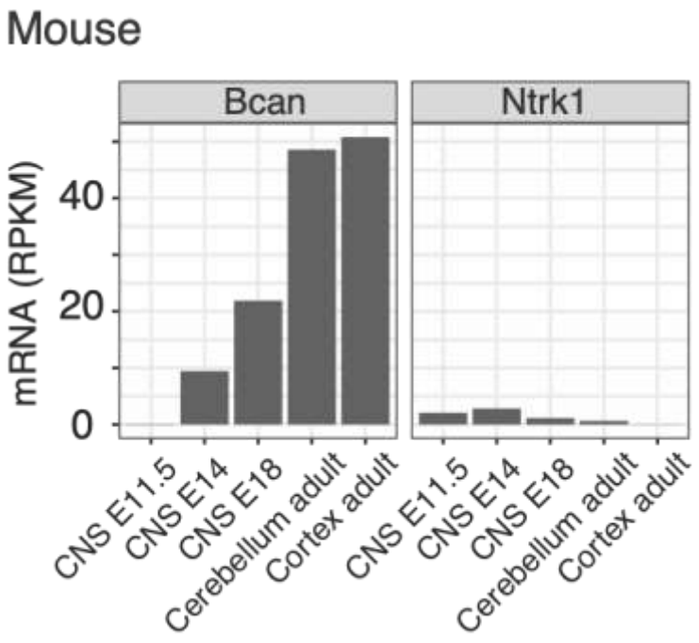
Oldrini B., Curiel A. et al., Nature Communications (2018)

BCAN-NTRK1 fusion



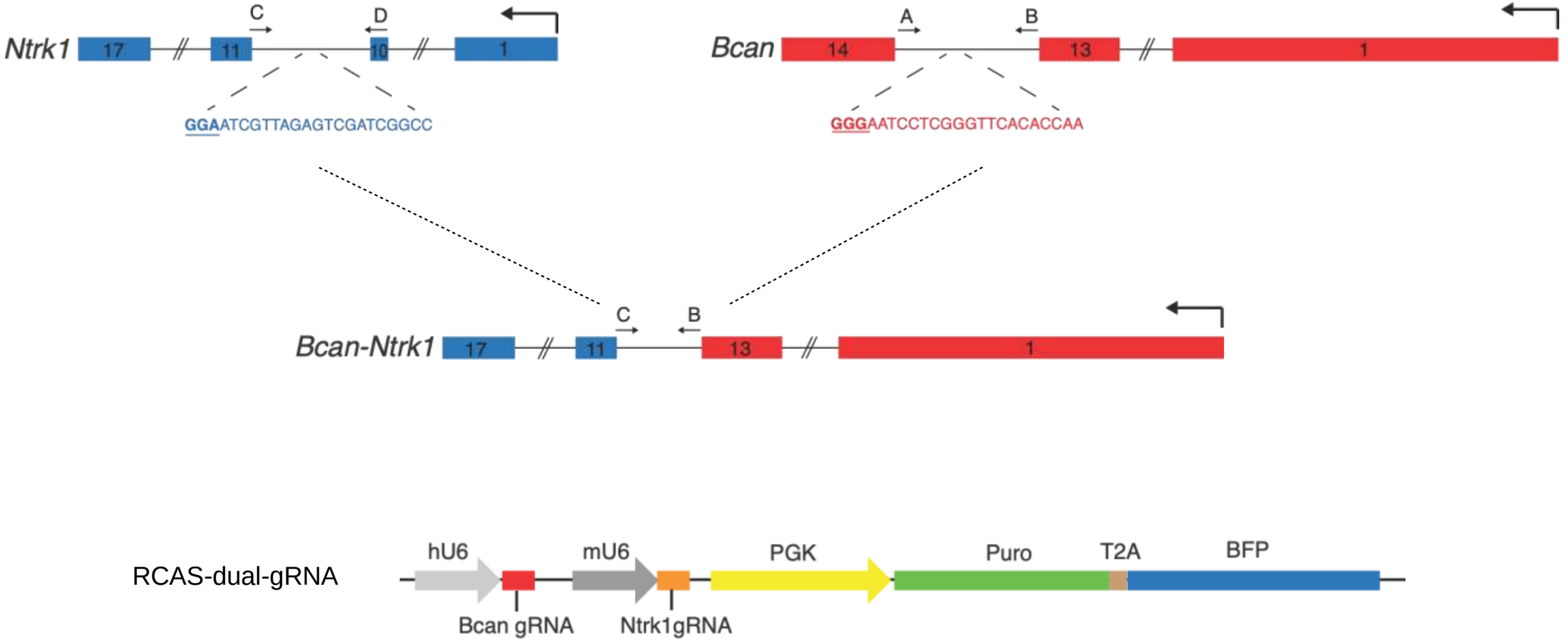






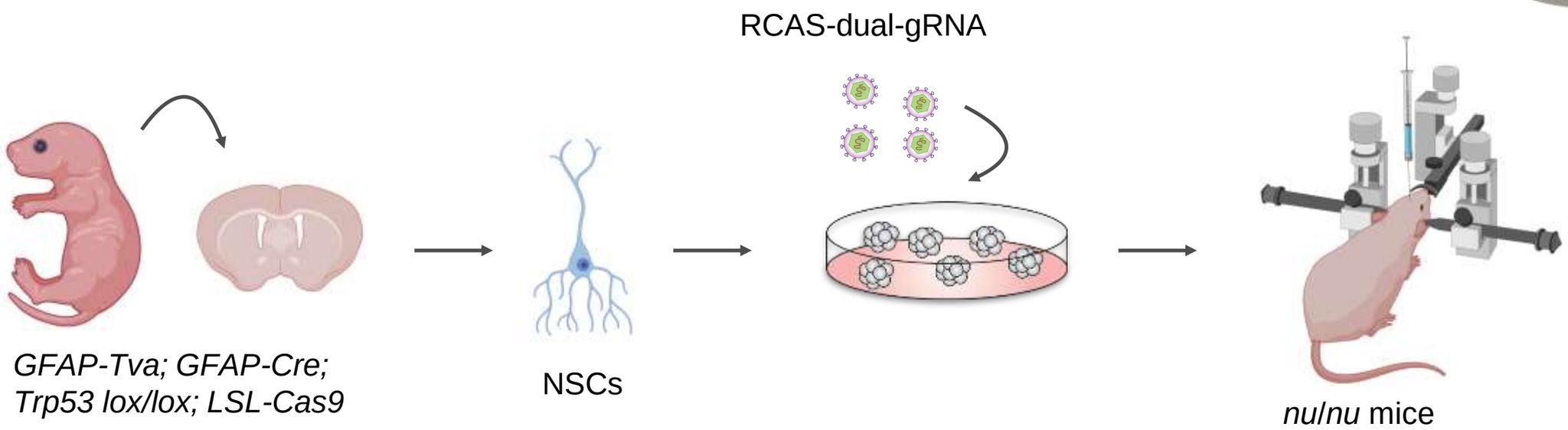
Oldrini B., Curiel A. et al., Nature Communications (2018)

Generation of the *Bcan-Ntrk1* in mouse cells

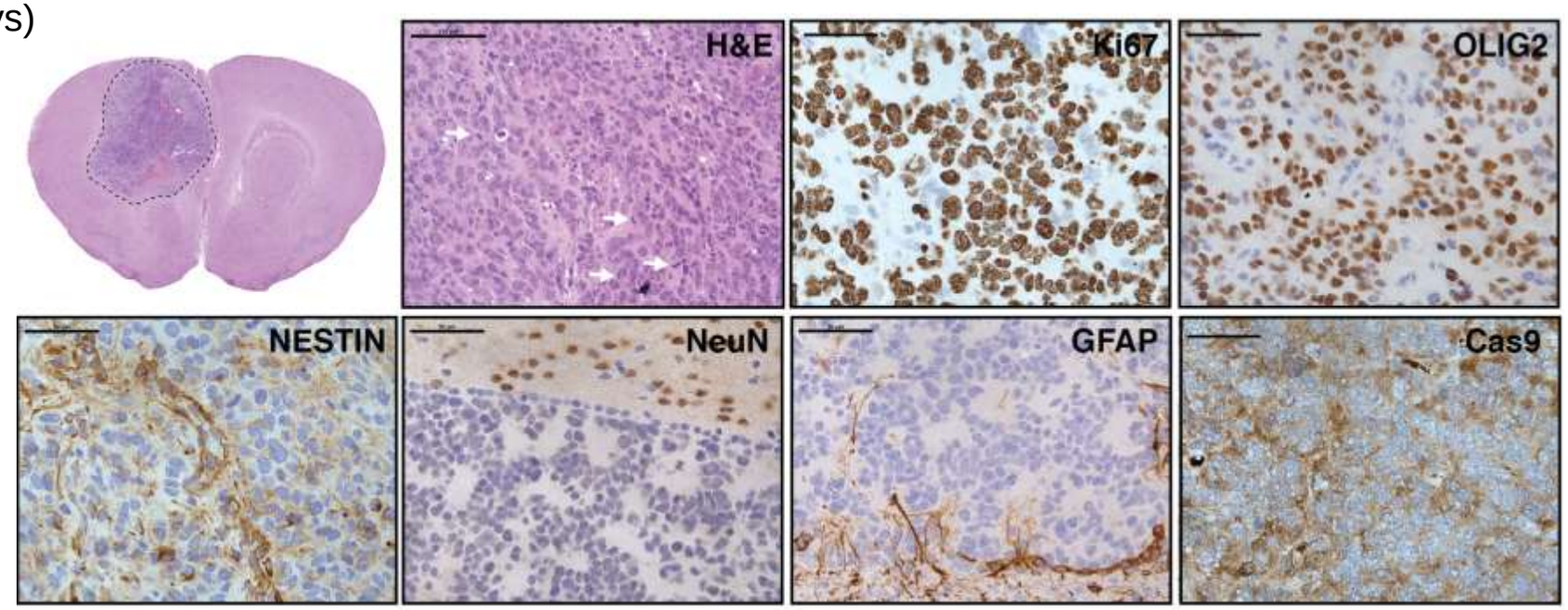


Oldrini B., Curiel A. et al., Nature Communications (2018)

Bcan-Ntrk1 induces high-grade gliomas



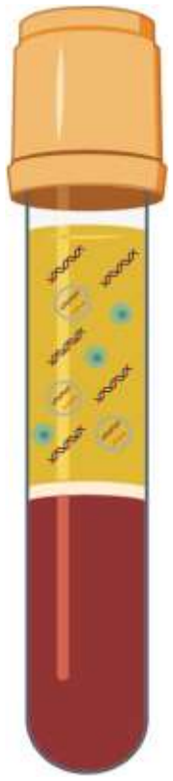
4/6 mice with tumor
(avg survival 72 days)



Oldrini B., Curiel A. et al., Nature Communications (2018)

Liquid biopsy in brain cancer

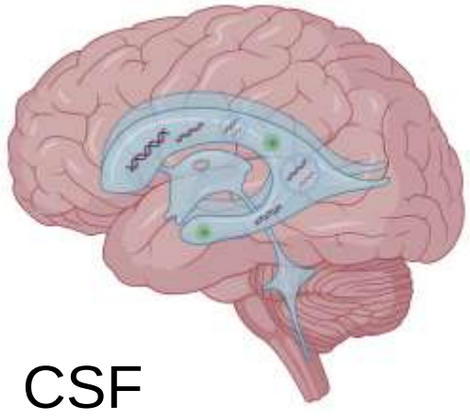
Blood



ctDNA

EVs

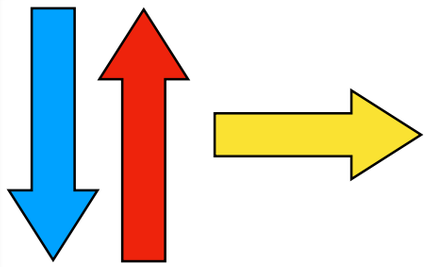
CTCs



CSF

Cancer detection

Screening or earlier diagnosis

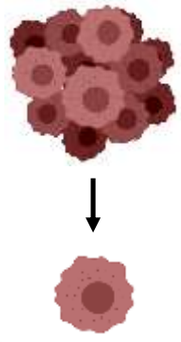


Monitoring response

Molecular profiling

Monitoring clonal evolution

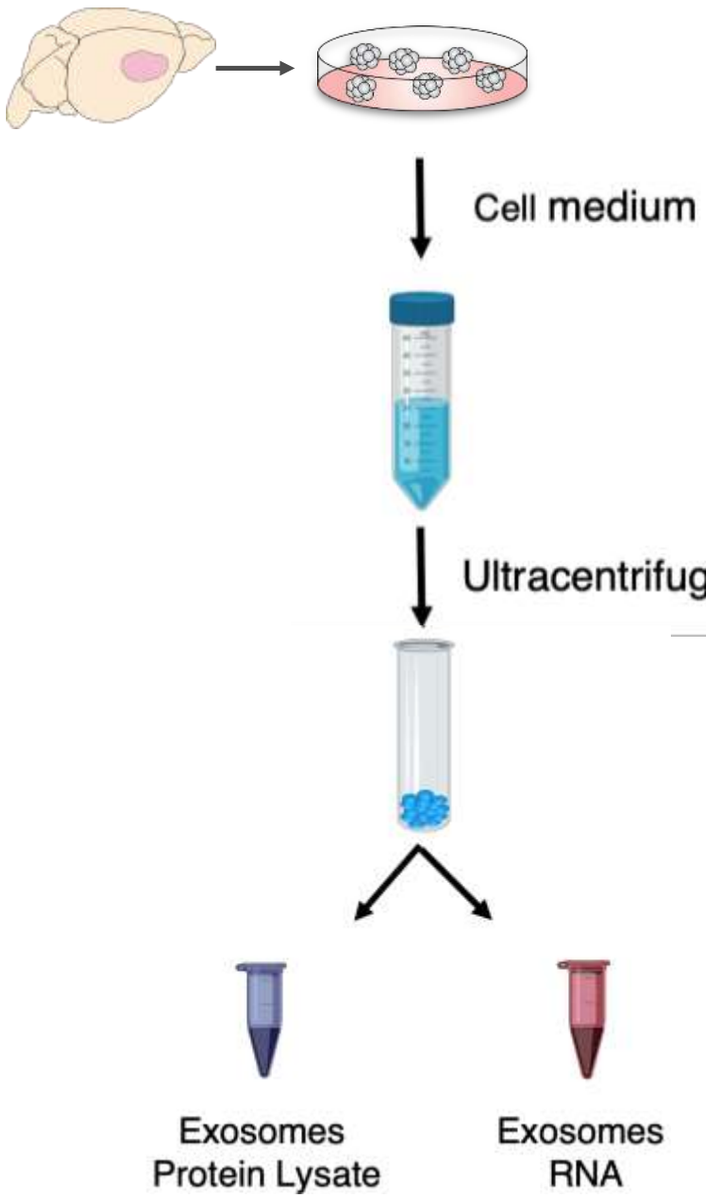
Treatment selection

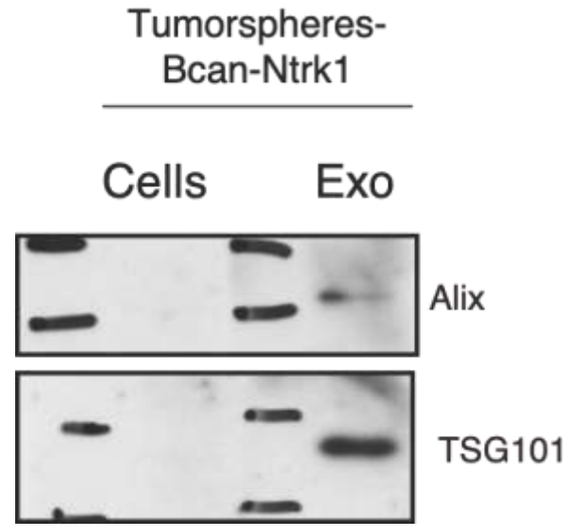


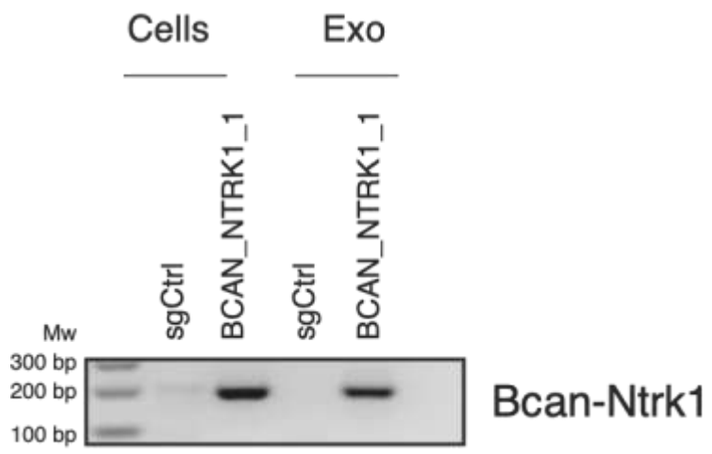
Detection of residual disease

adapted from Rosenfeld, AACR 2019

Bcan-Ntrk1 fusion is enriched in tumor-derived exosomes

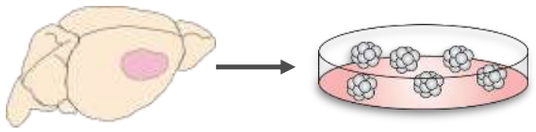


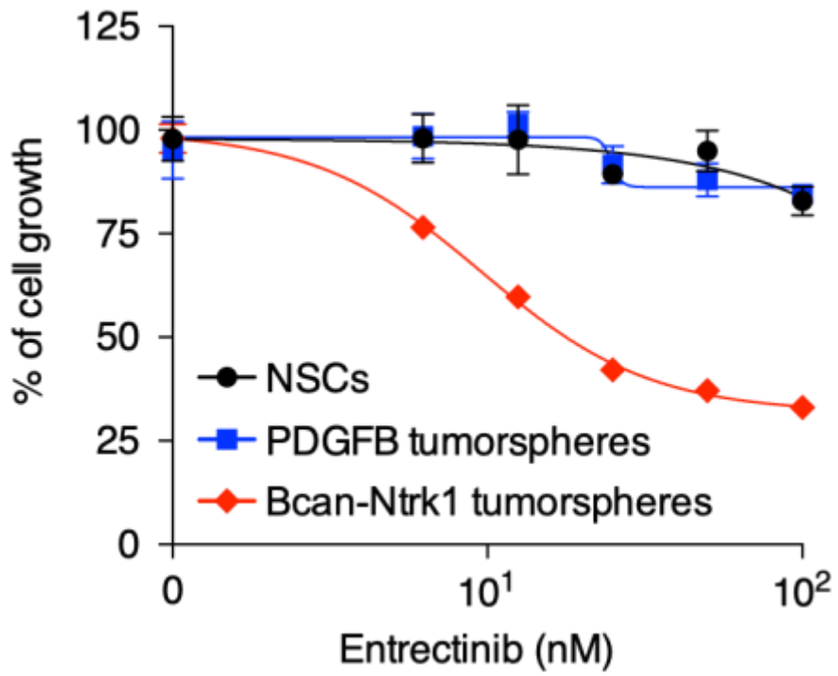


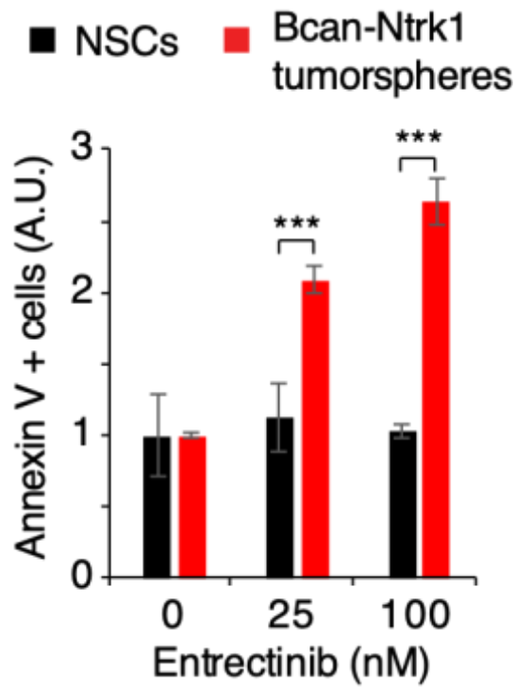


Oldrini B et al., unpublished

Bcan-Ntrk1 gliomas are sensitive to Trk inhibition

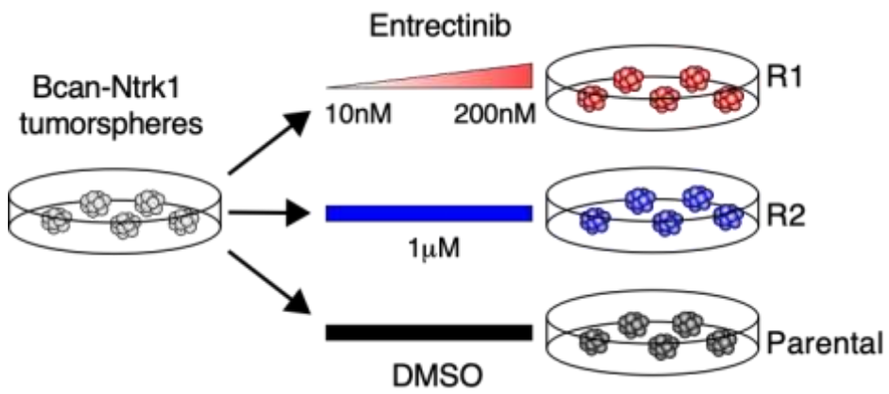


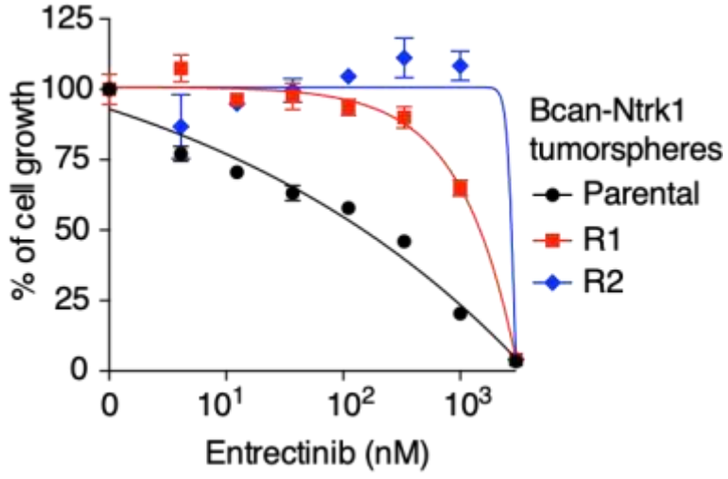


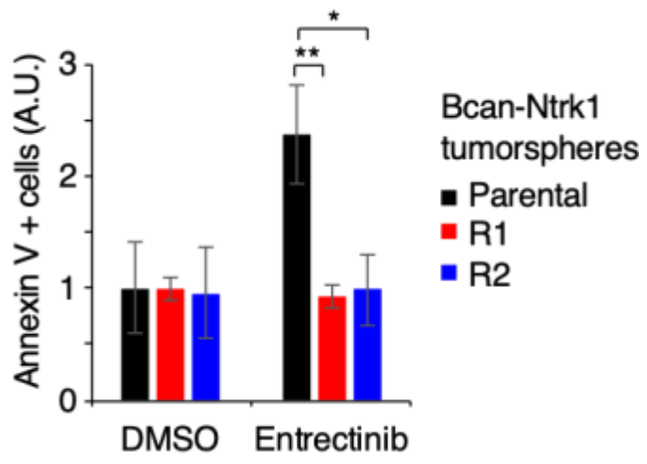


Oldrini B., Curiel A. et al., Nature Communications (2018)

Modelling acquired resistance to TRK inhibitors



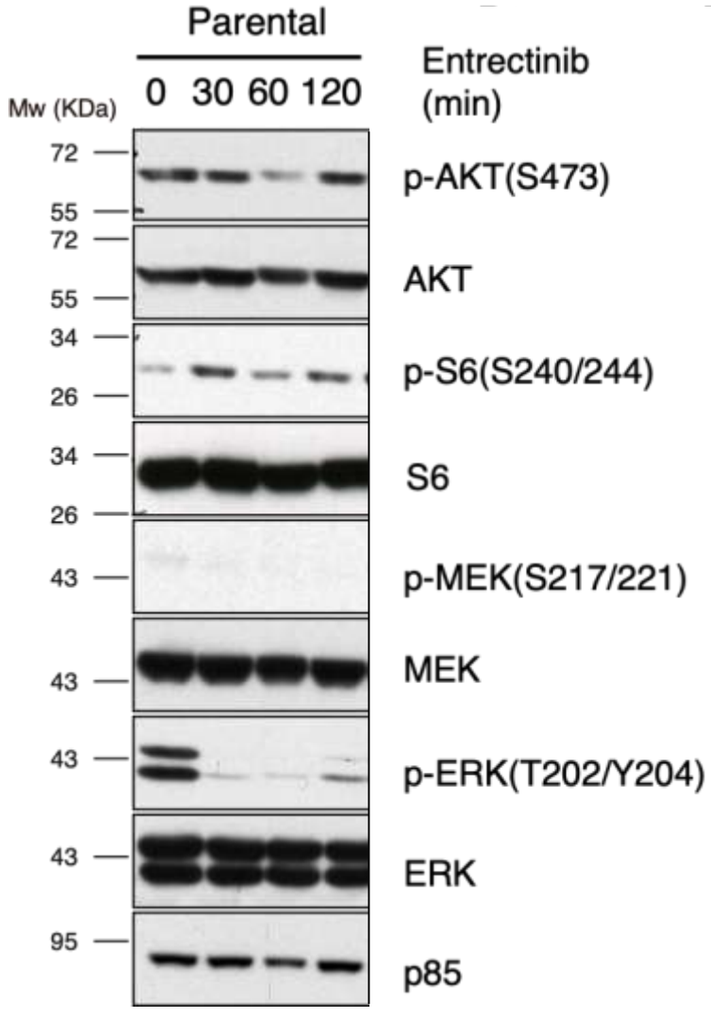


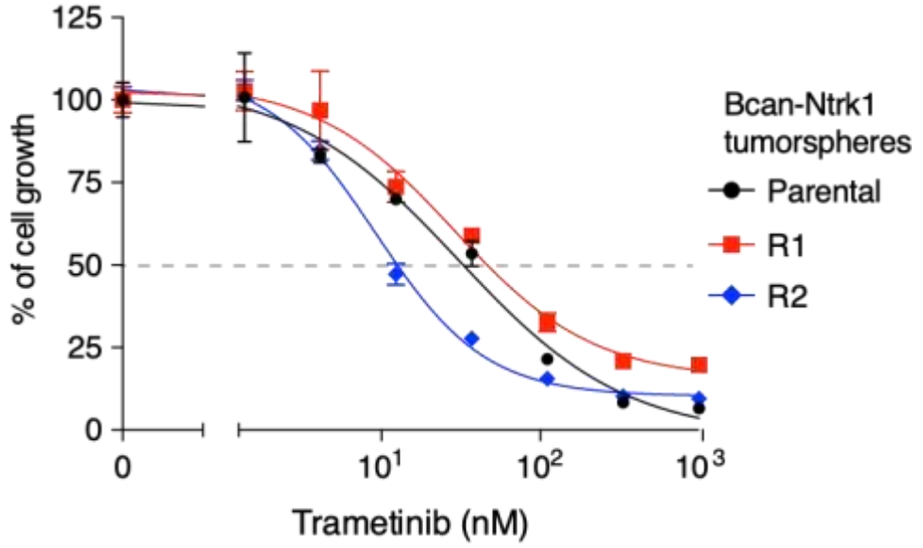


NO mutations in the kinase domain

Oldrini B., Curiel A. et al., Nature Communications (2018)

MAPKi overcomes Entrectinib resistance





Oldrini B., Curiel A. et al., Nature Communications (2018)

MAPK pathway alterations lead to TRKi resistance

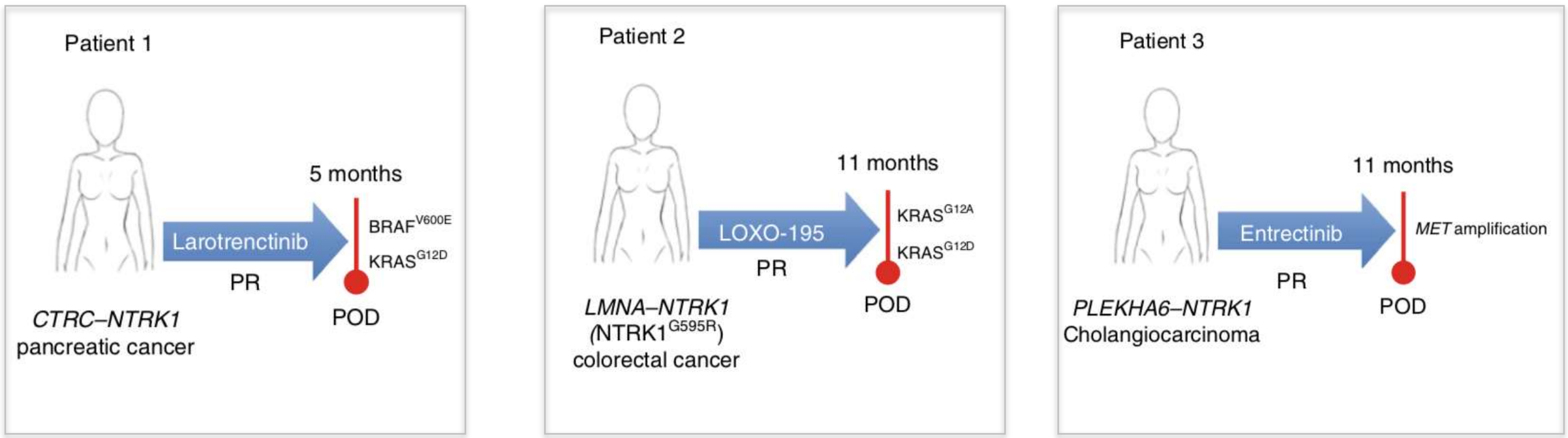
LETTERS
https://doi.org/10.1038/s41591-019-0542-z
nature medicine

Resistance to TRK inhibition mediated by convergent MAPK pathway activation

Emiliano Cocco[1,2,11], Alison M. Schram[3,4,11], Amanda Kulick[5,6], Sandra Misale[5], Helen H. Won[7], Rona Yaeger[3,5], Pedram Razavi[1,3], Ryan Ptashkin[2], Jaclyn F. Hechtman[2], Eneda Toska[1], James Cownie[1], Romel Somwar[1,2], Sophie Shifman[1,2], Marissa Mattar[5,6], S. Duygu Selçuklu[8], Aliaksandra Samoila[8], Sean Guzman[5,6], Brian B. Tuch[9], Kevin Ebata[9], Elisa de Stanchina[5,7], Rebecca J. Nagy[10], Richard B. Lanman[10], Brian Houck-Loomis[7], Juber A. Patel[7], Michael F. Berger[1,2,7], Marc Ladanyi[1,2], David M. Hyman[3,4], Alexander Drilon[3,4*] and Maurizio Scaltriti[1,2*]

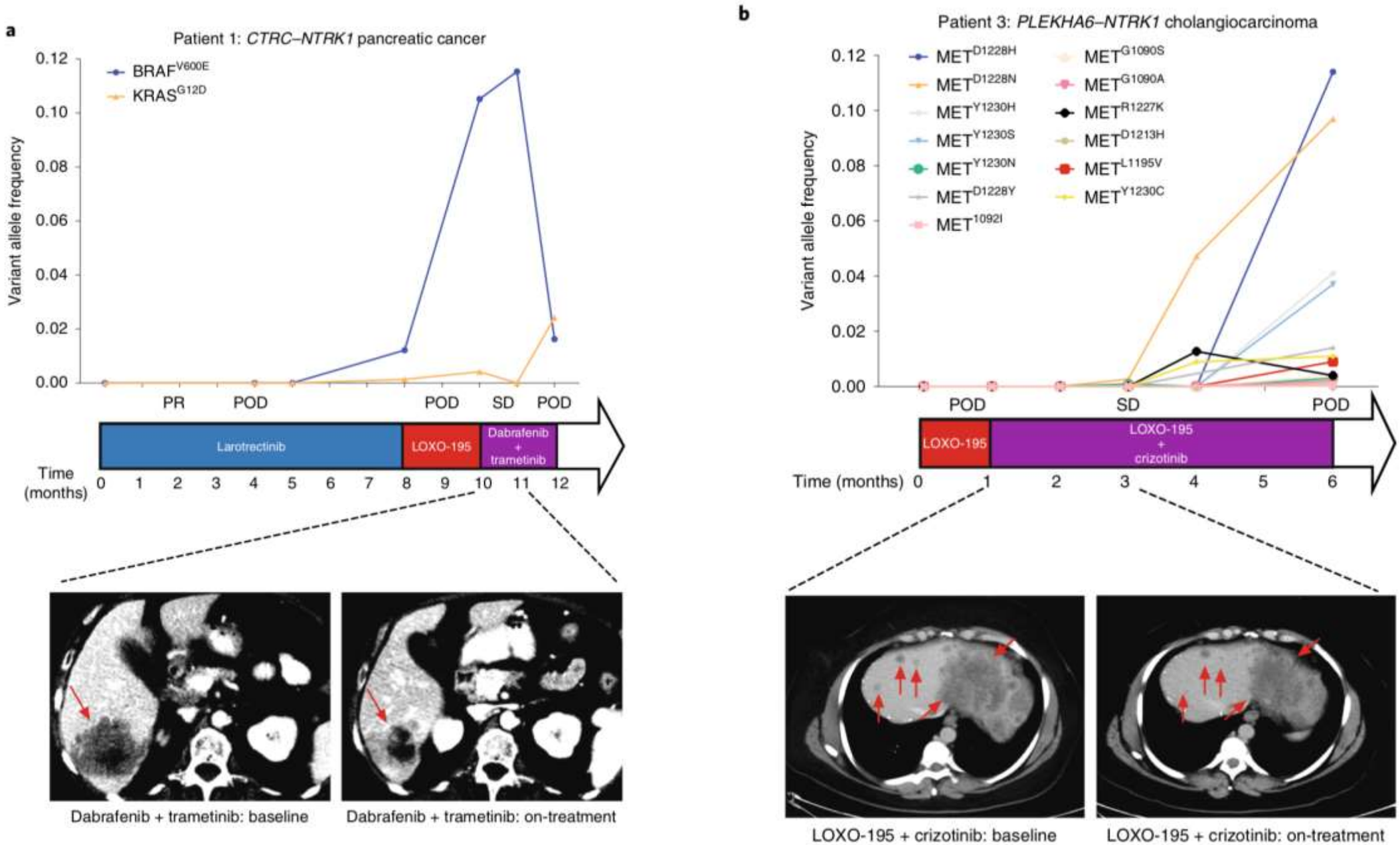
Cocco E et al. (Sept 25th, 2019)

MAPK pathway alterations lead to NTRKi resistance



Cocco E et al. (2019)

Combination treatment for NTRKi-resistant tumors



Cocco E et al. (2019)

Conclusions

- TRK inhibition as an agnostic tumour treatment for NTRK fusion positive patients

- Solvent front mutations as main mechanism of TRKi resistance

- MAPK activation (KRASmut, BRAFmut, MET AMP) is emerging as an alternative mechanism of resistance to both 1st and 2nd generation TRK inhibitors

- Liquid biopsy is a potential approach for detection of NTRK fusion at mRNA level

Acknowledgments

Seve-Ballesteros F. Brain Tumor Group

Massimo Squatrito (Principal Investigator)
Barbara Oldrini (Staff Scientist)
Miguel Jimenez Alcazar (Post Doc)
Veronica Matia (PhD Student)
Maria del Mar Gardeazabal (PhD Student)
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Claudia Troncone
Nuria Vaquero
Marcos Galan Ganga
Lucas Kissing
Carolina Marqués

Mic&Met Group
Hector Peinado
Susana Garcia

Genomic Unit
Orlando Domínguez

Animal Facility
Miriam Garcia
Flora Diaz
Gema Iglesias

Funding:

Acknowledgments

Cancer Cell Biology Programme
Erwin Wagner
Latifa Bakiri
Özge Uluçkan
Paco Real
Nabil Djouder

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Entrectinib resistance in *ROS1*-rearranged NSCLCs

Investigational New Drugs
https://doi.org/10.1007/s10637-019-00795-3

PRECLINICAL STUDIES

Entrectinib resistance mechanisms in *ROS1*-rearranged non-small cell lung cancer

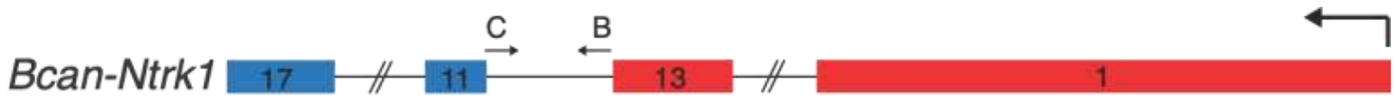
Bo Mi Ku[1] · Yeon Hee Bae[1] · Kyoung Young Lee[1] · Jong-Mu Sun[2] · Se-Hoon Lee[2] · Jin Seok Ahn[2] · Keunchil Park[2] · Myung-Ju Ahn[2]

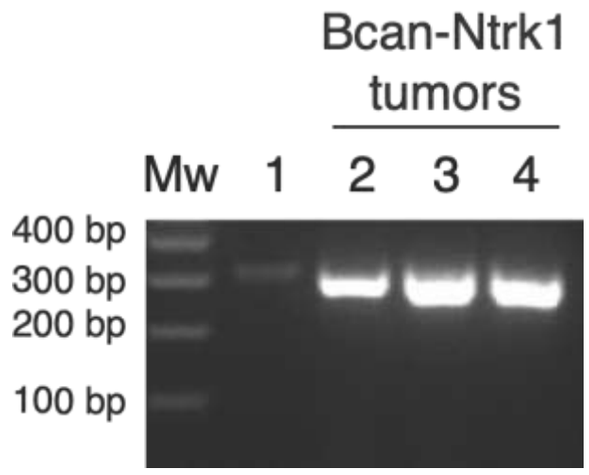
Received: 11 March 2019 / Accepted: 15 May 2019

Here, we characterized the molecular basis of resistance in ***entrectinib-resistant ROS1-rea***

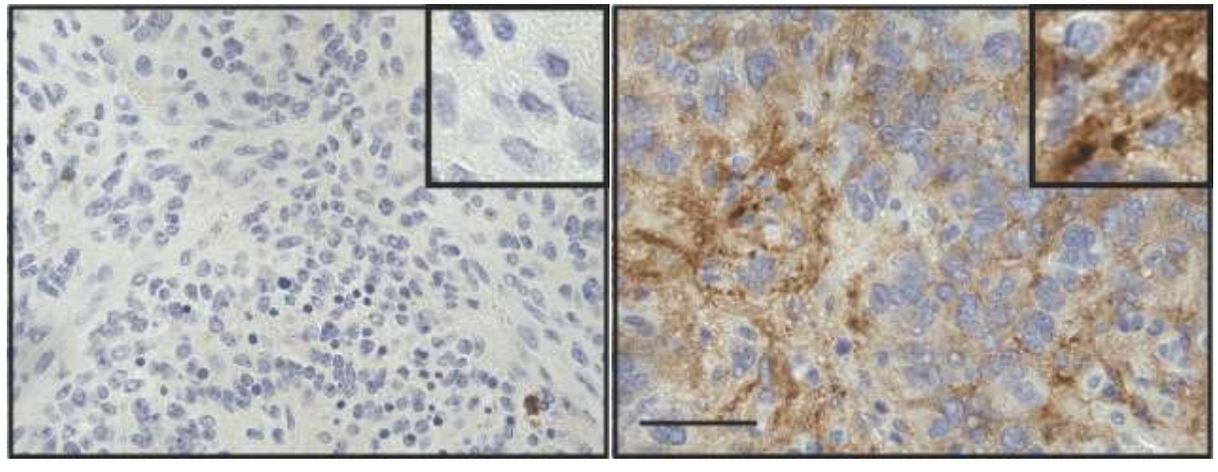
Cocco E et al. (2019)

Bcan-Ntrk1 gliomas express TrkA





pan-Trk HIC

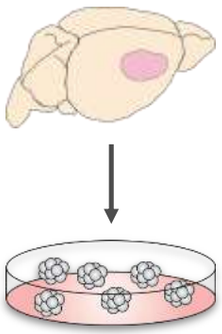


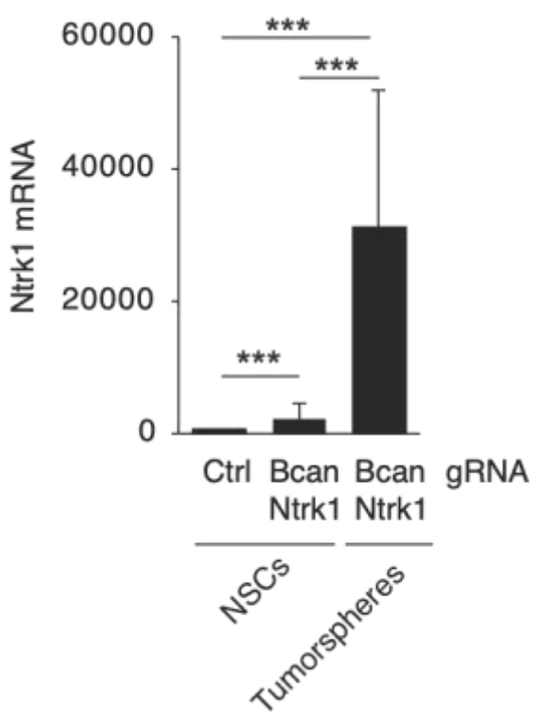
PDGFB

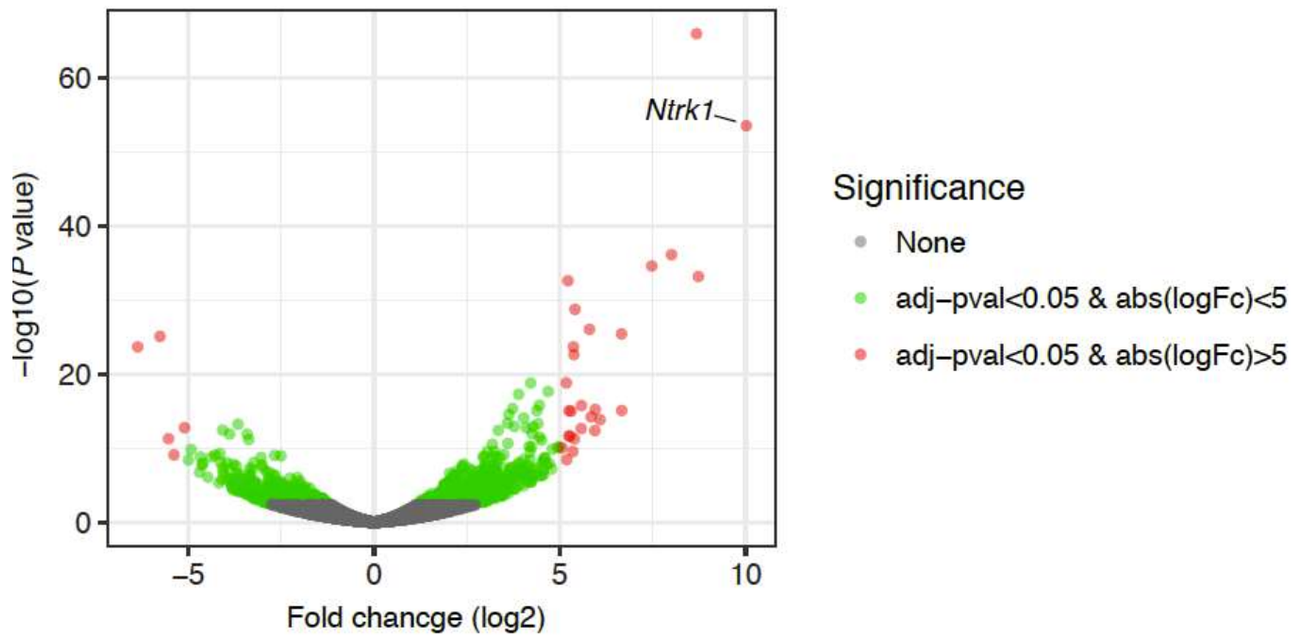
Bcan-Ntrk1

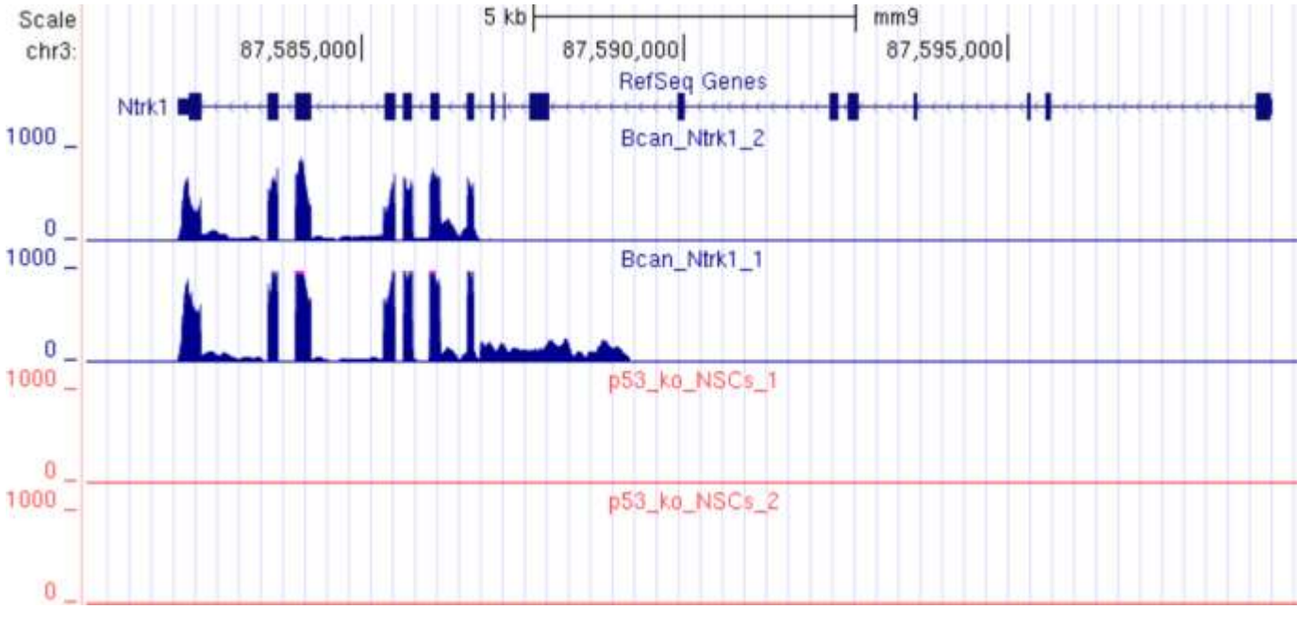
Oldrini B., Curiel A. et al., Nature Communications (2018)

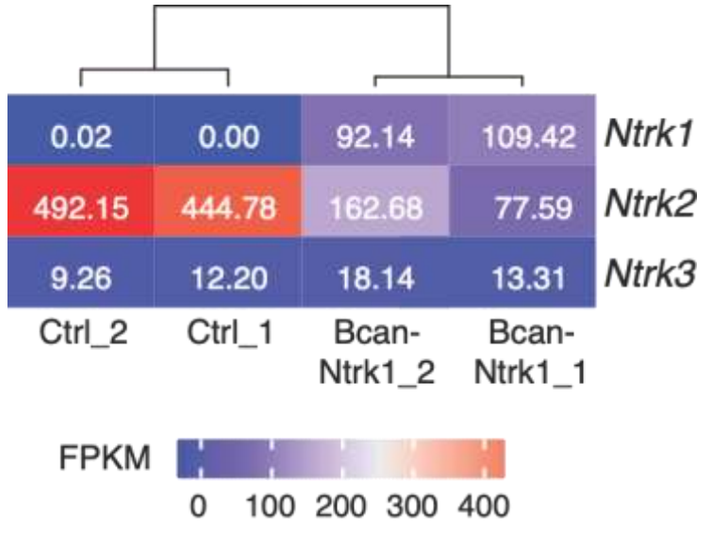
High Ntrk1 levels drives tumor formation







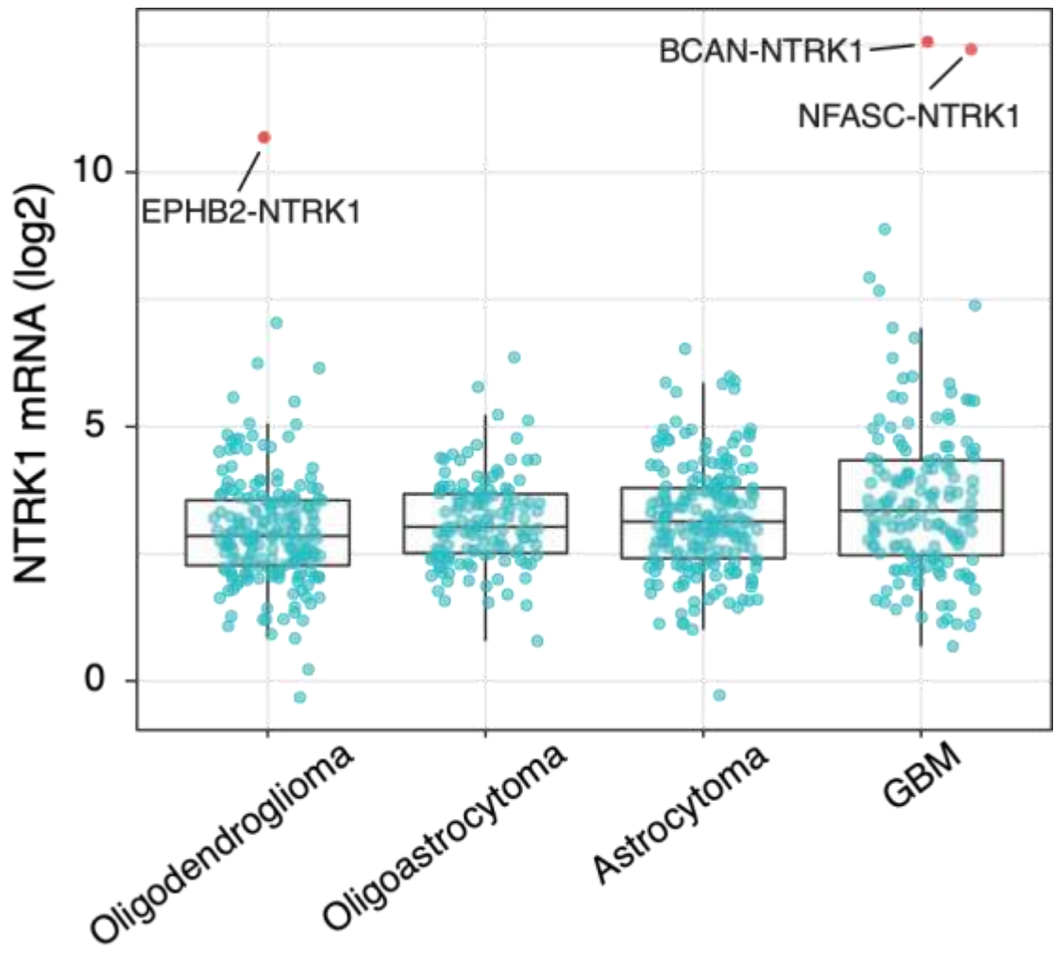




Oldrini B., Curiel A. et al., Nature Communications (2018)

High *NTRK1* levels as possible drivers of glioma tumor formation

TCGA LGG-GBM dataset

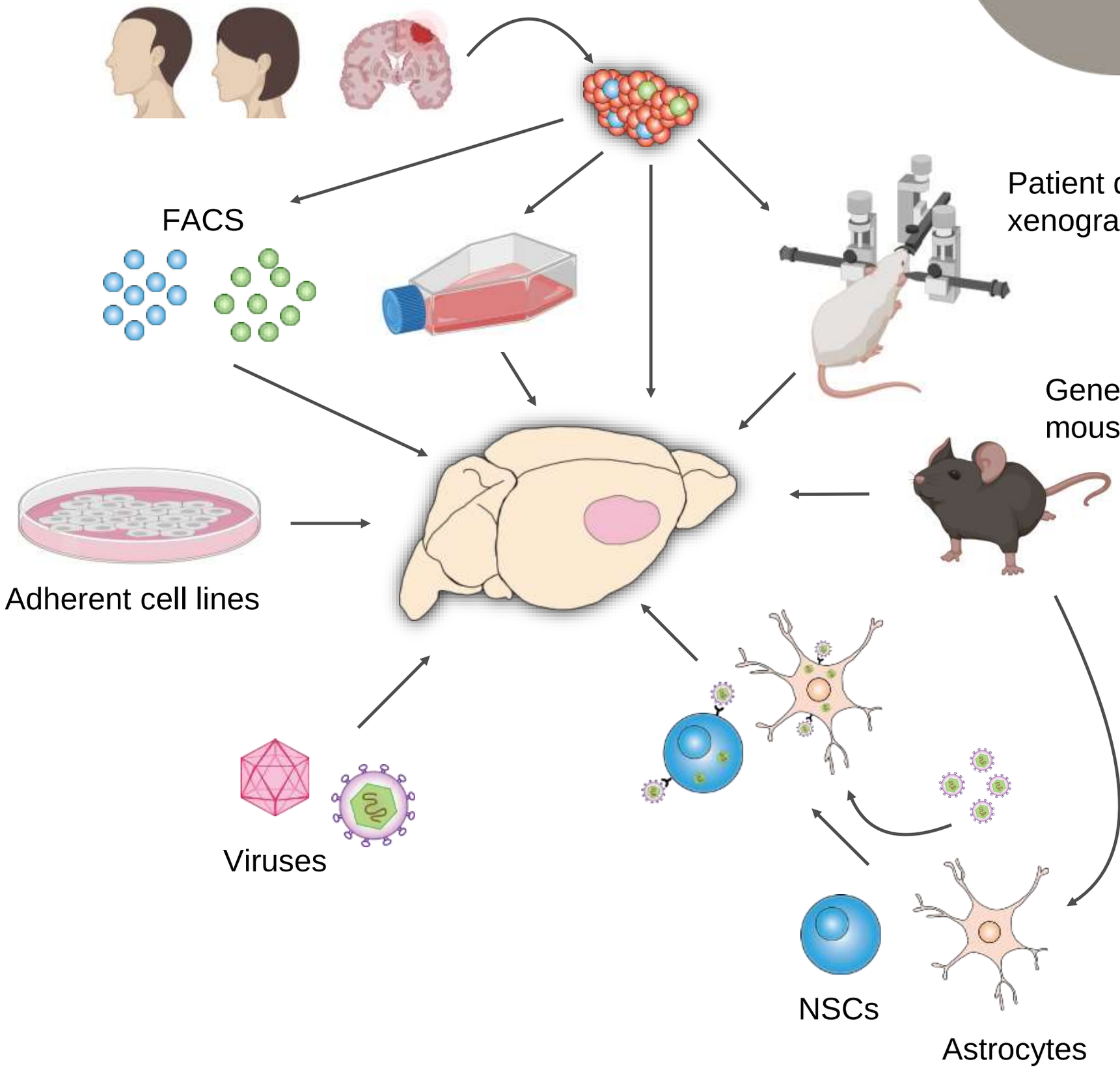


Oldrini B., Curiel A. et al., Nature Communications (2018)

cnio stop cancer

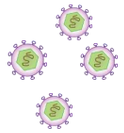
Are they all oncogenic?

Glioma in the lab: a story of mice and men

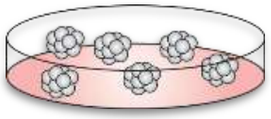


Generation of the *Bcan-Ntrk1* in mouse NSCs

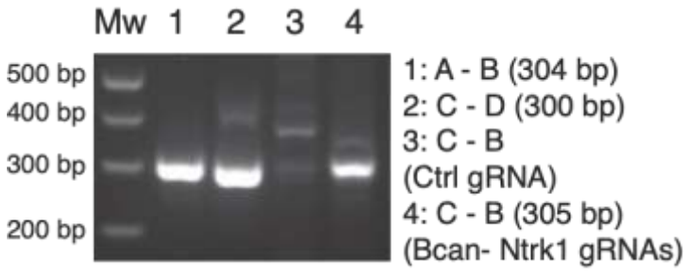
RCAS-dual-gRNA

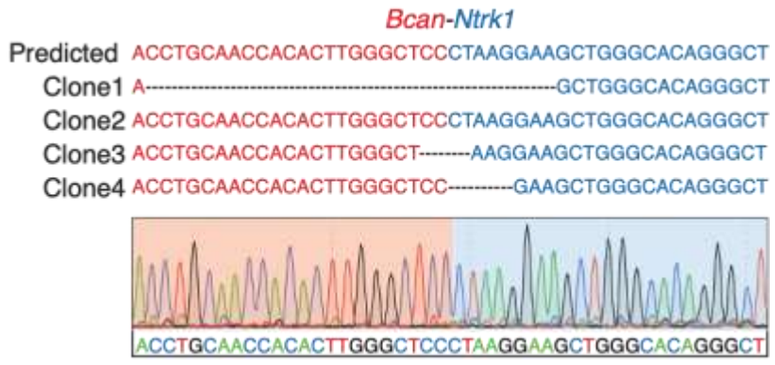


GFAP-Tva; GFAP-Cre;
Trp53 lox/lox; LSL-Cas9

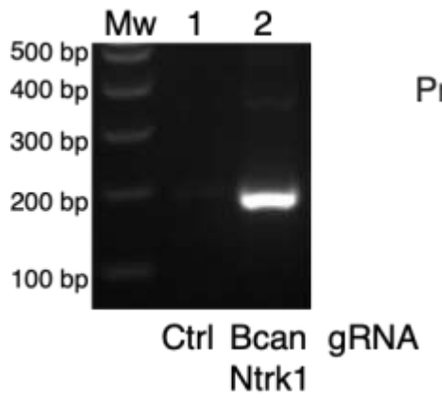


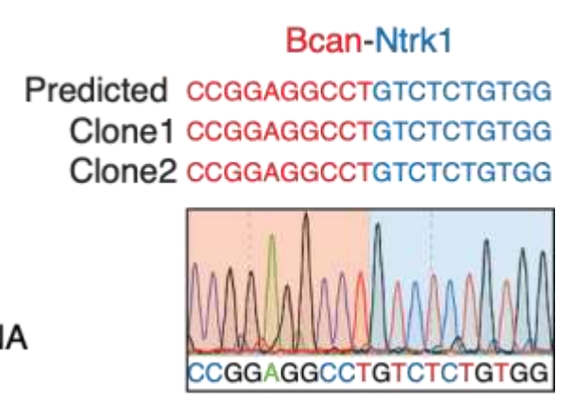
Genomic DNA





mRNA

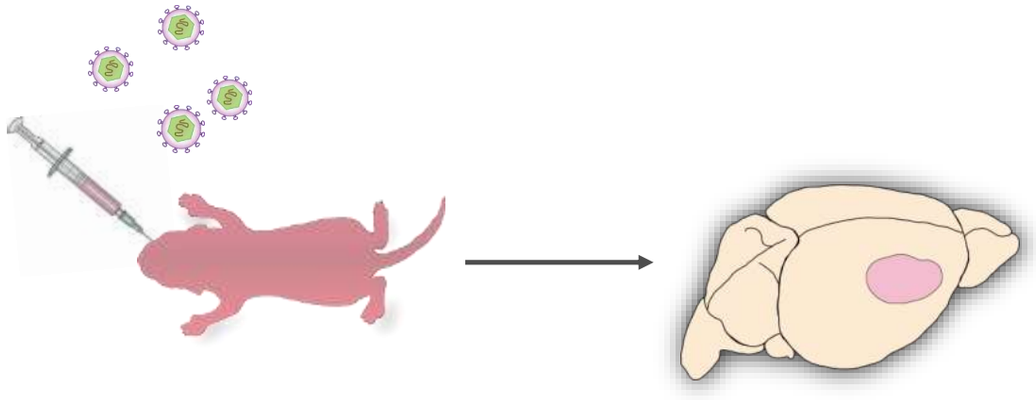




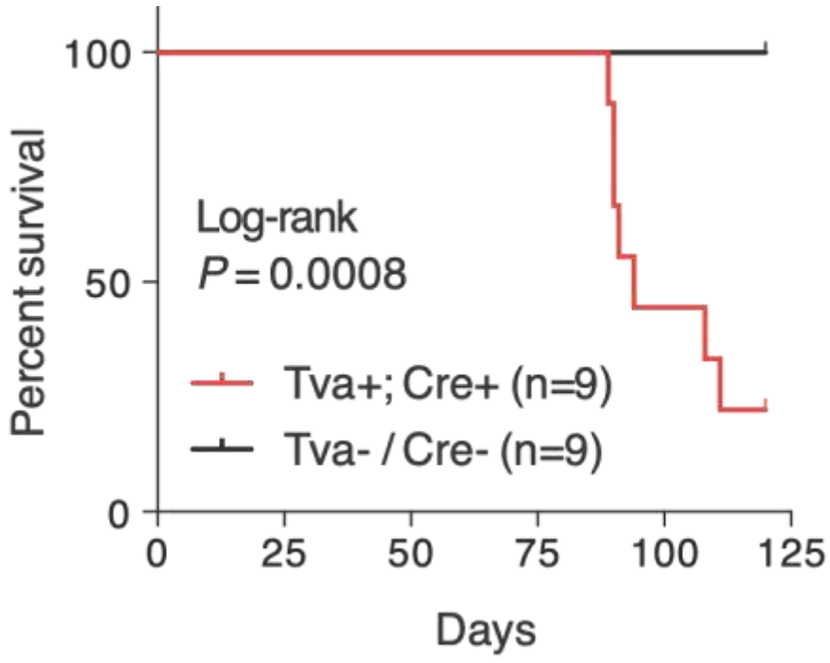
Oldrini B., Curiel A. et al., Nature Communications (2018)

Bcan-Ntrk1 induces high-grade gliomas

RCAS-dual-gRNA



GFAP-Tva; GFAP-Cre;
Trp53 lox/lox; LSL-Cas9



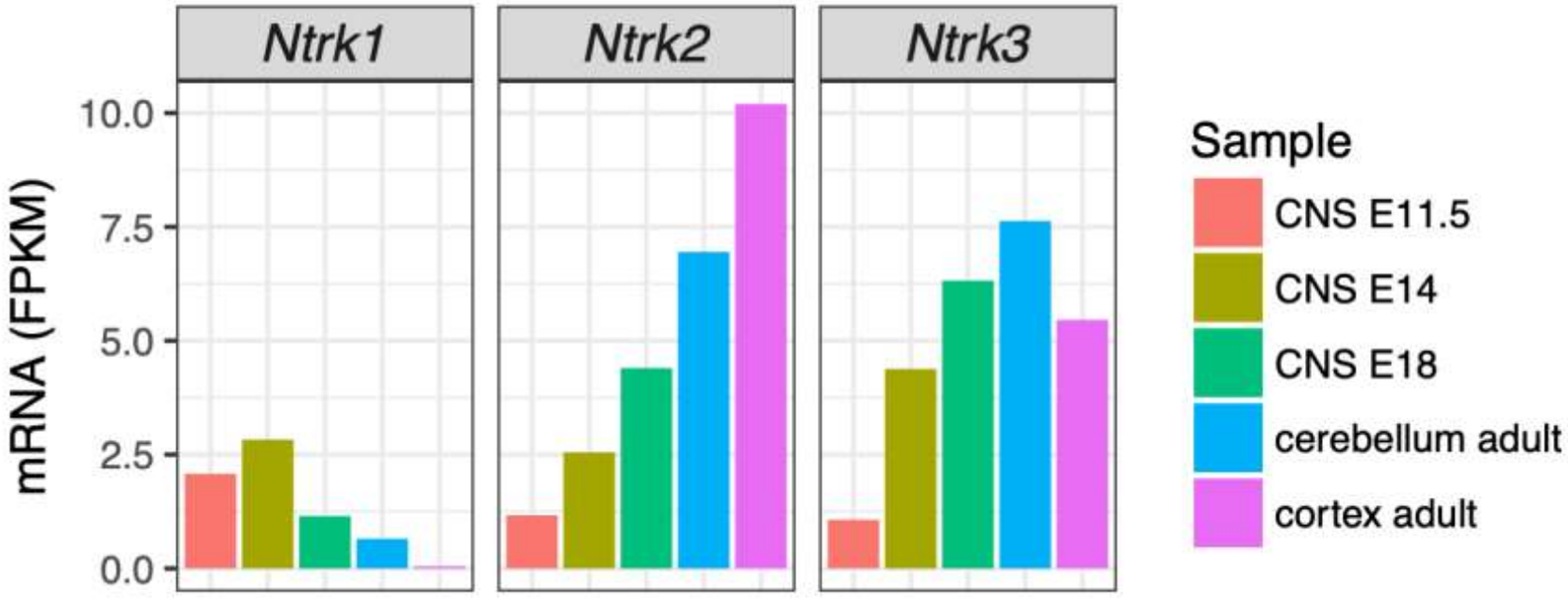
Oldrini B., Curiel A. et al., Nature Communications (2018)

Ongoing work

NTRK (*NTRK1*, *NTRK2* and *NTRK3*) gene fusions have been identified in multiple types of gliomas:

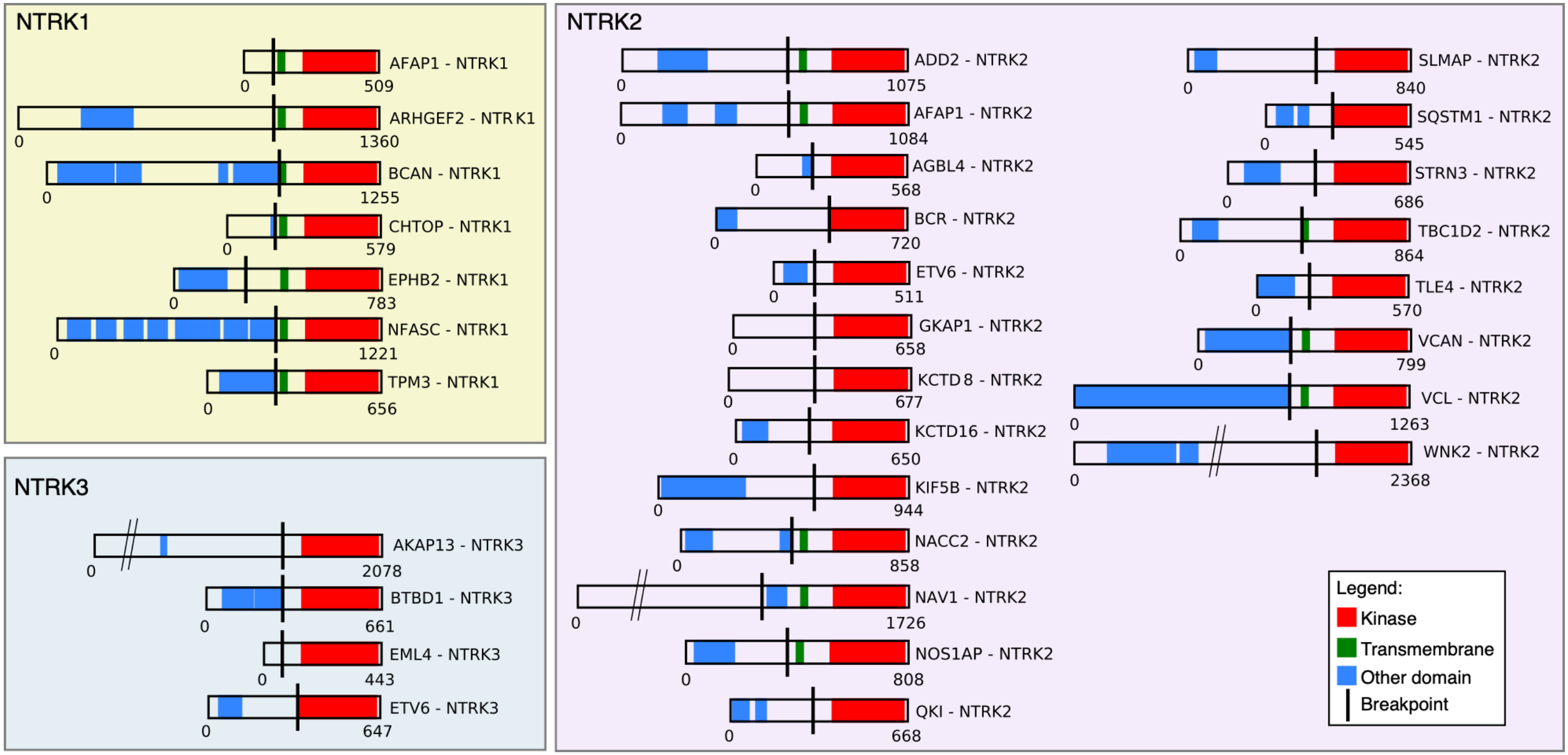
- Are they all oncogenic?
- How do they drive tumorigenesis (overexpression, ligand-independent activation, etc.)?
- Can we target them?
- Do they have equal response to TRKi?
- Can we detect them using liquid biopsy?

Temporal expression of NTRK genes



Oldrini B et al., unpublished

NTRK fusion in gliomas

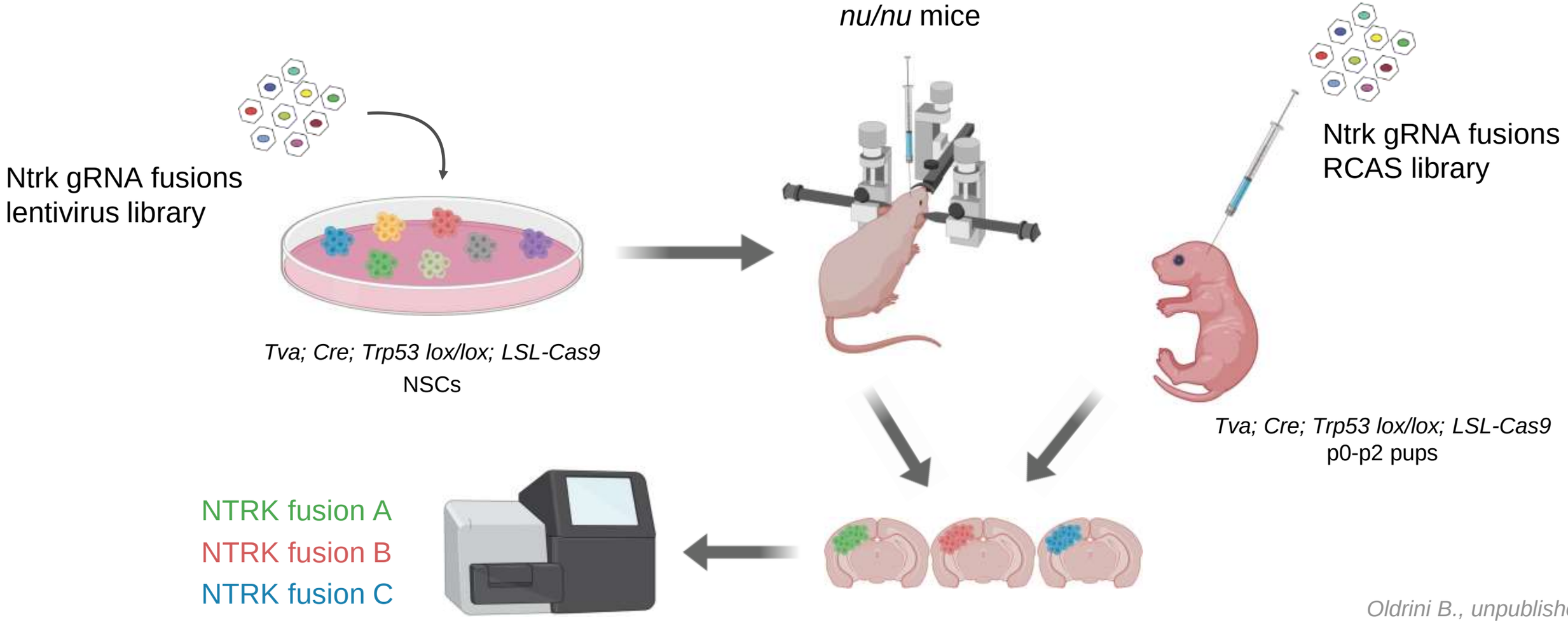


Oldrini B et al., unpublished

NTRK *In vivo* dual-gRNA screening

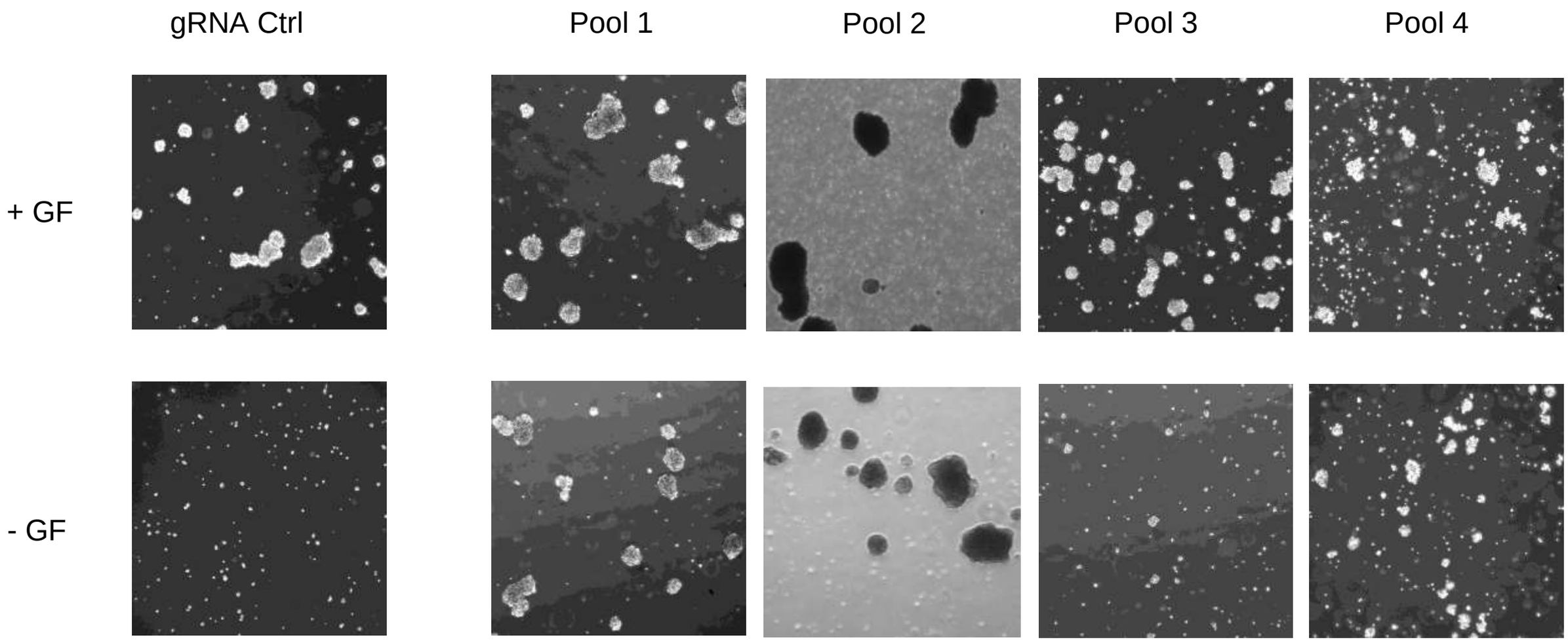
Ntrk fusions gRNA library:

- 32 different NTRK gene fusions
- 3 gRNAs per gene (9 pairs)
- Approximately 350 gRNAs (7 pools)



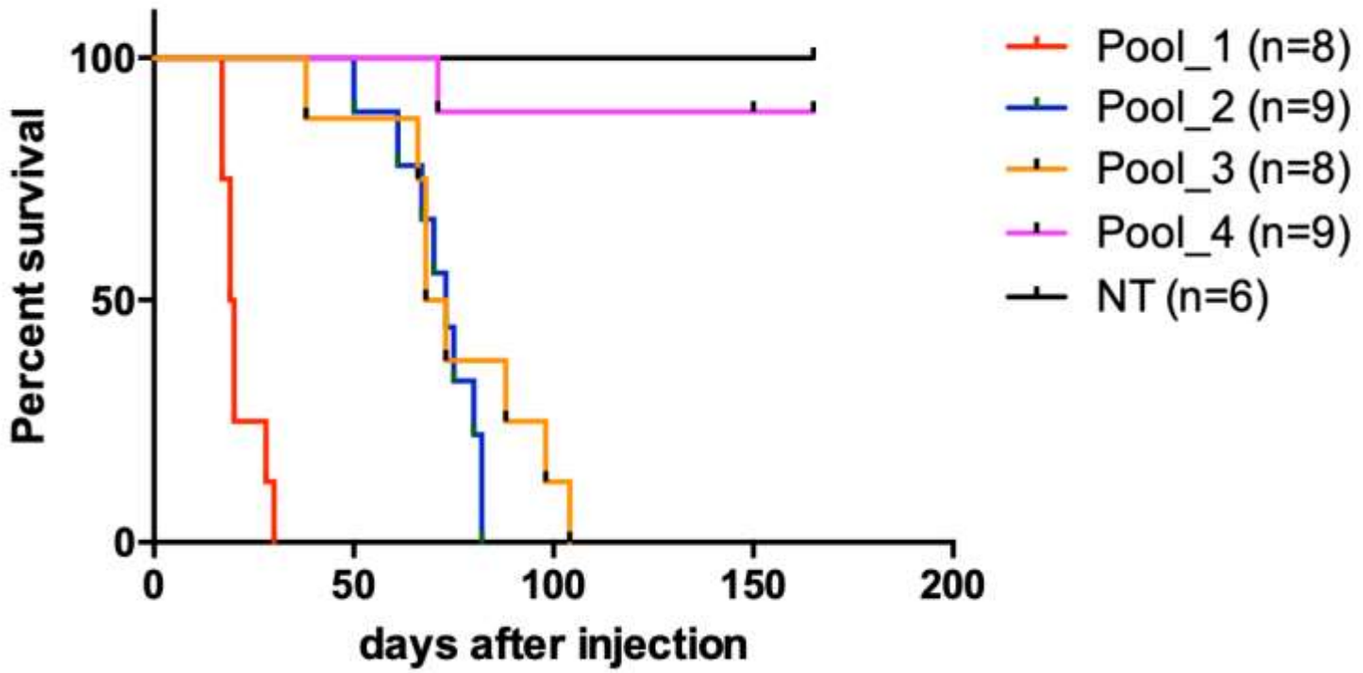
Oldrini B., unpublished

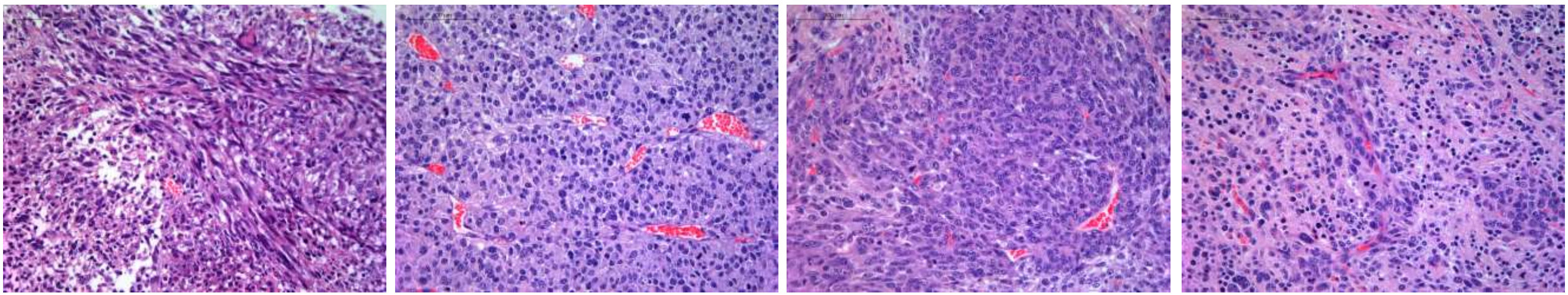
NTRK fusions lead to growth factor independence



Oldrini B., unpublished

NTRK fusions lead to aggressive high-grade gliomas





Oldrini B., unpublished

NTRK fusions: discovery timeline

Identification of nerve growth factor (NGF), the first neurotrophin

Identification of TRKA, TRKB and TRKC as high-affinity neurotrophin receptors

Purification of brain-derived neurotrophic factor (BDNF)

Identification of neurotrophin 3 (NT-3)

Identification of neurotrophin 4 (NT-4)

Severe neuropathies developed by *Ntrk* knockout mice

BDNF–TRKB pathway involvement in neuroblastoma progression

Loss-of-function *NTRK1* mutations identified in patients with congenital insensitivity to pain with anhidrosis (CIPA)

Data emerge implicating the involvement of TRK signalling in ovulation

Crystal structure of NGF in complex with TRKA determined

First activating TRKA alternative variant identified

TRKB downregulation associated with hyperphagia and hyperdipsia in mice

Crystal structures of the kinase domains of TRKA and TRKB determined

1950s | 1982 | 1989–1991 | 1993–1994 | 2000 | 2010 | 2015 | 2017 | 2018

Identification of *NTRK* as an oncogene: *TPM3–NTRK1* found in a human colorectal carcinoma

Identification of *NTRK1* fusions in papillary thyroid carcinoma

Identification of the first *NTRK3* fusion (*ETV6–NTRK3*) in infantile fibrosarcoma

Identification of *NTRK2* fusions in pilocytic astrocytoma

First-generation TRK inhibitors enter clinical trial testing

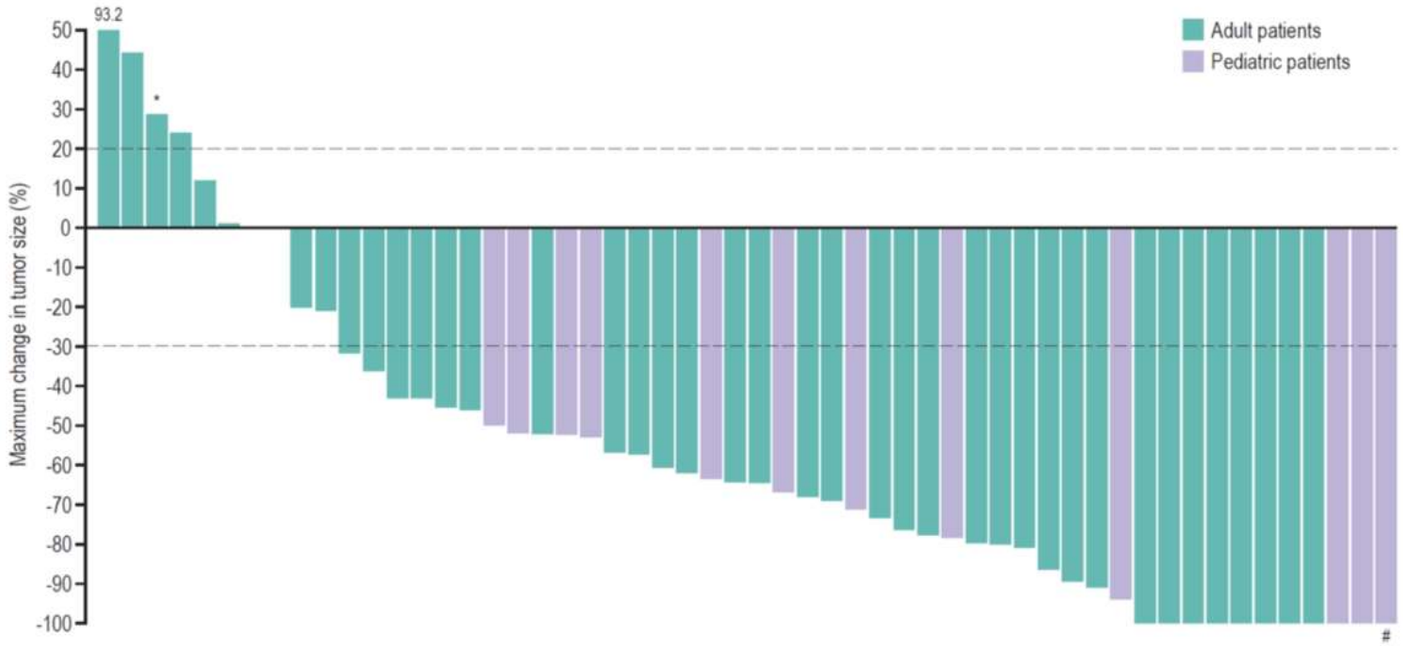
Second-generation TRK inhibitors enter clinical trial testing

Larotrectinib achieves histology-agnostic and age-agnostic responses in *NTRK* fusion-positive solid tumours

Larotrectinib and entrectinib receive FDA breakthrough designation for the treatment of *NTRK* fusion-positive solid tumours

Cocco E et al. (2018)

Age independent efficacy of Larotrectinib



Drilon et al., N Engl J Med (2018)