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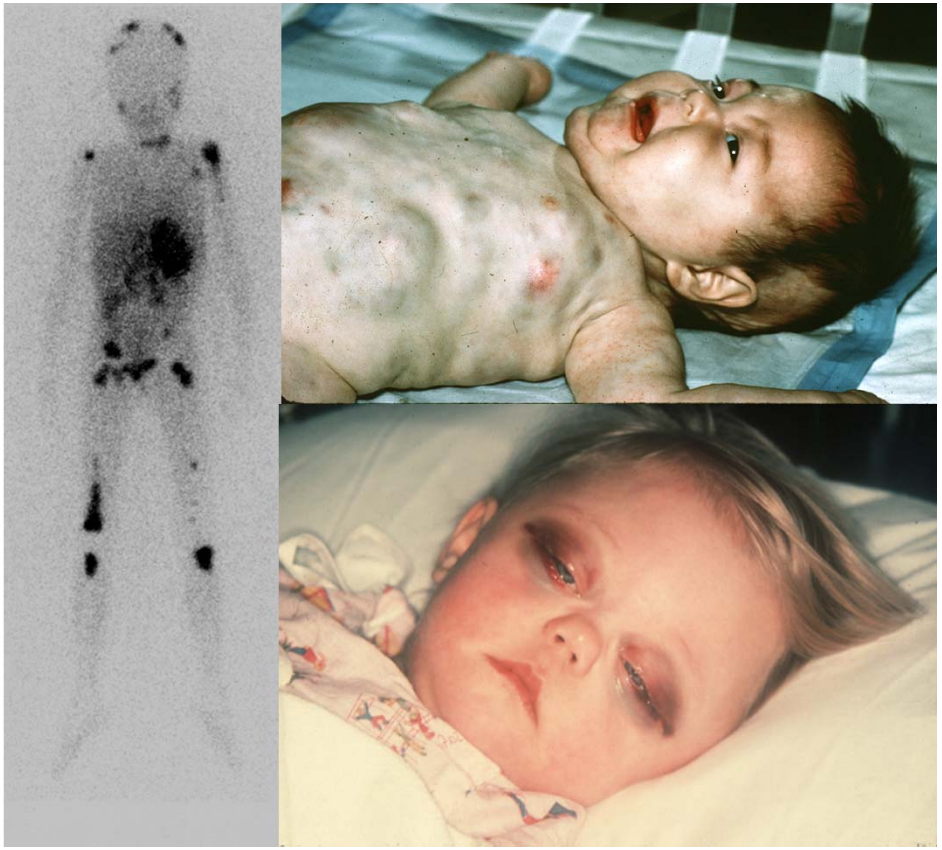
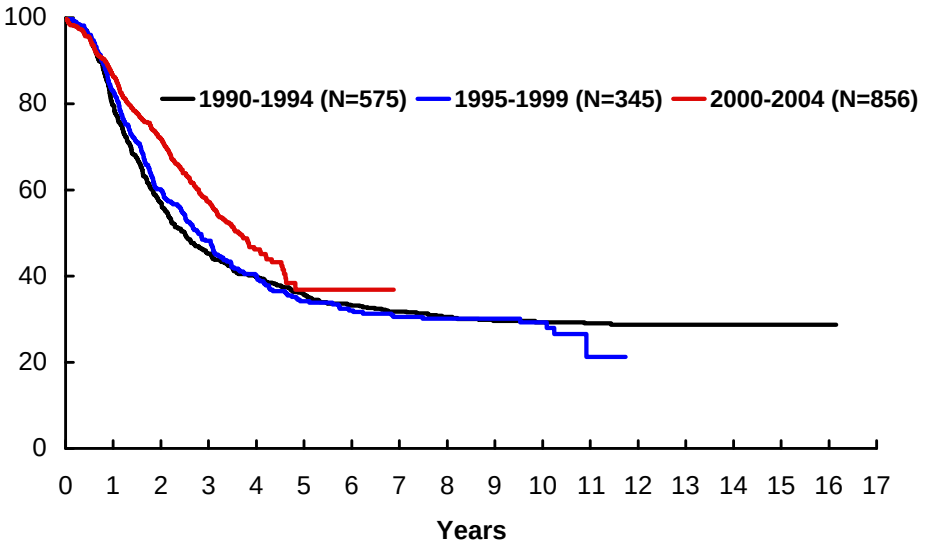
Neurblastoma-TARGET: Motivation

- **Important pediatric problem**
  - **15% of childhood cancer mortality**
  - **50% of cases metastatic and highly malignant at diagnosis**
  - **Cure rates stagnant over last two decades**
    - **Despite dramatic intensification of treatment intensity**
    - **Survivors with significant morbidity**

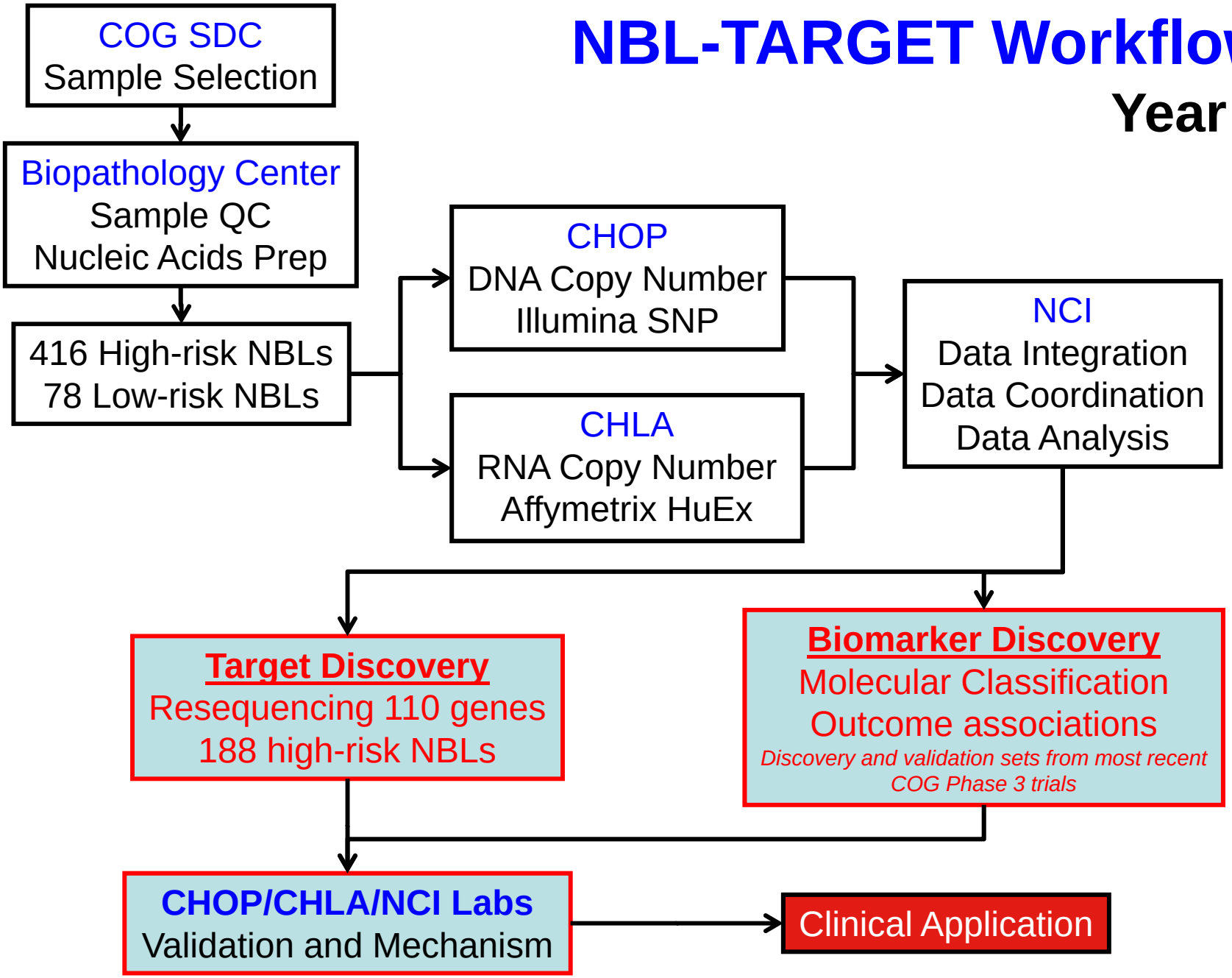


- **Neuroblastoma genomics highly predictive of clinical course**
  - **Recurrent amplification (MYCN) and deletions (1p36 and 11q23) used by COG to stratify therapy**
  - **But….no bona-fide and tractable molecular targets known**



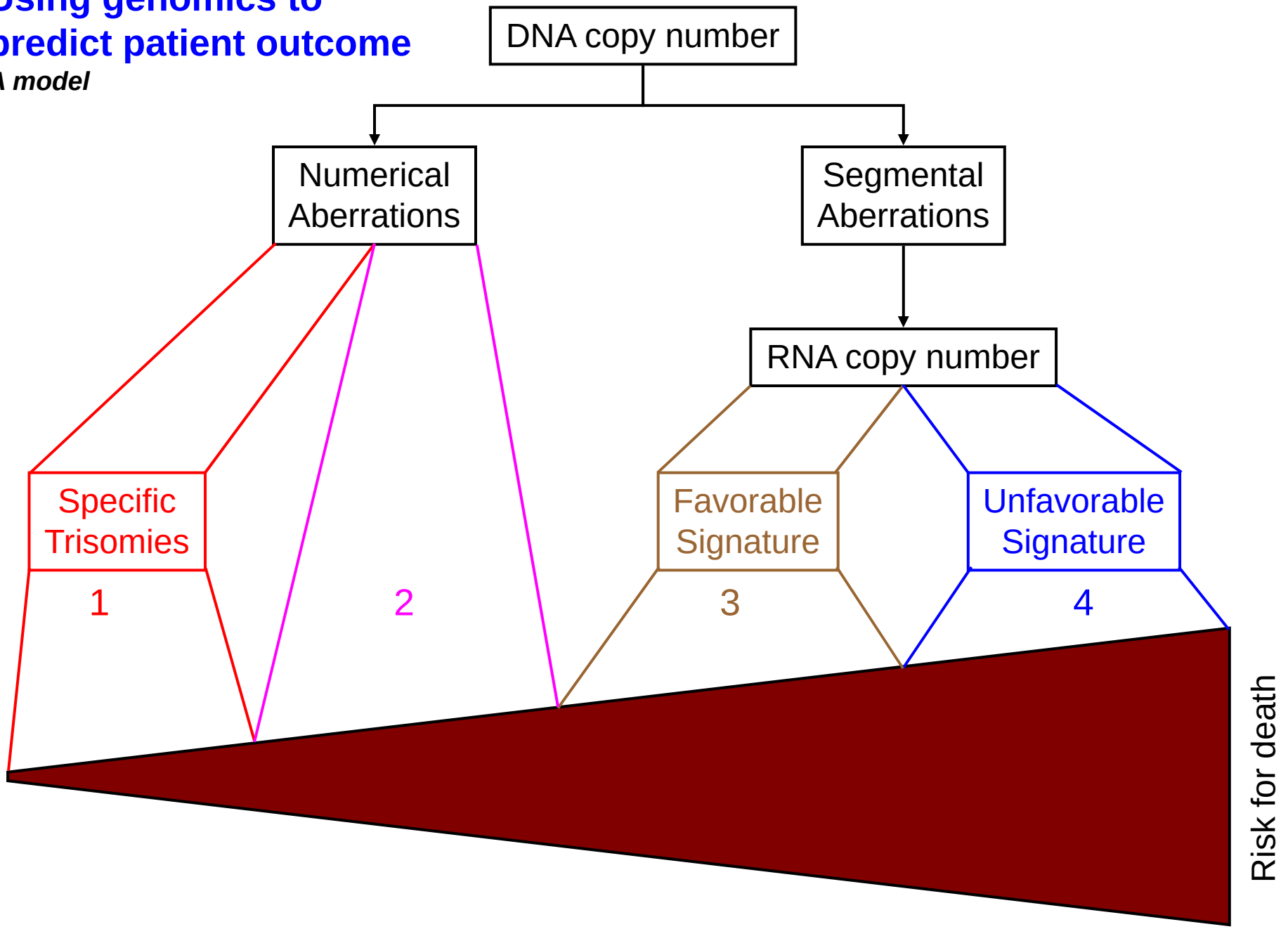
NBL-TARGET Workflow

Year 1



Using genomics to predict patient outcome

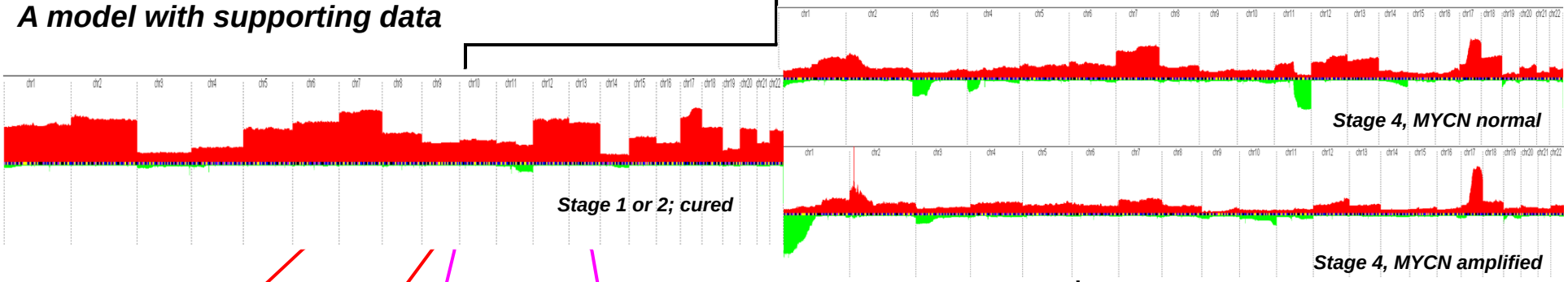
A model



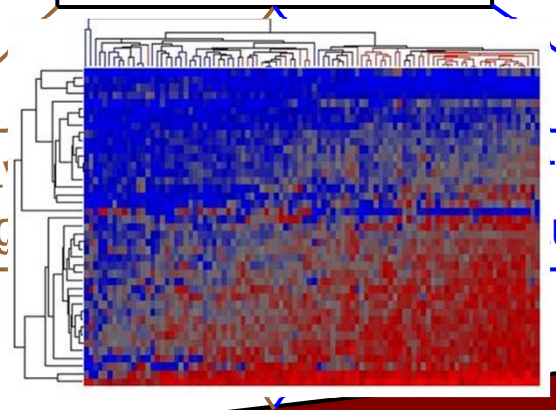
Using genomics to predict patient outcome

A model with supporting data

DNA copy number



RNA copy number



Specific Trisomies

1

Chr.	p value
1	**p = 0.005**
2	**p = 0.009**
5	**p = 0.005**
7	**p = 0.040**
1, 2, and 5	**p = 0.002**
Hyperdiploid	**p = 0.572**
Diploid	

Fav… Sig…

…able …ure

Risk for death

Discovering mutated targets

Gene resequencing selection criteria

- Genes within regions of copy number aberration
  - Homozygous deletion
  - Absolute loss and/or LOH
  - Relative gain (above the cell's DNA index)
  - Amplification
- Genes with differential gene expression
- Genes supported by the literature
- Genes with known mutations in other cancers (COSMIC database)
- Most candidates supported by two or more criteria

NBL-TARGET Resequencing Summary

- 188 samples
- 117 genes and microRNA sequenced
  - 1,066 exons
  - 1,591 amplicons
  - 1.11 Mb (0.037% of genome)
  - 679,862 traces generated so far

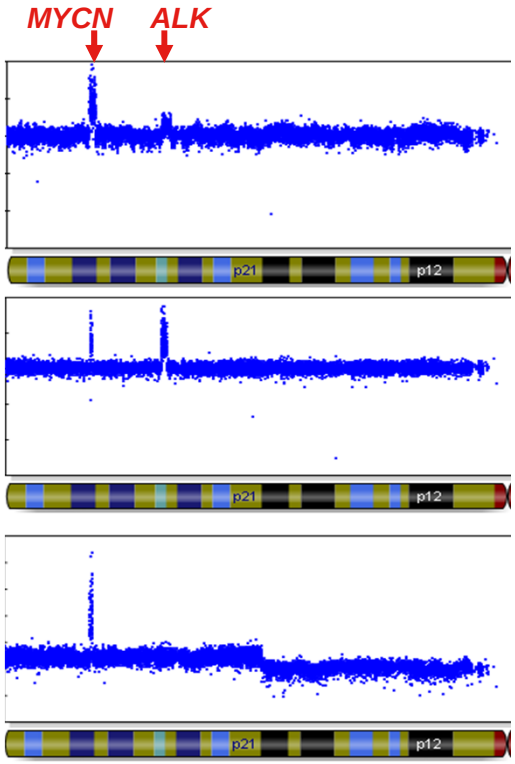
	Known	Novel	Total
frameshift	**1**	**47**	**48**
nonsense	**2**	**9**	**11**
proteinDel	**0**	**13**	**13**
proteinIns	**0**	**3**	**3**
splice	**1**	**12**	**13**
missense	**146**	**392**	**538**
silent	**244**	**275**	**519**
UTR_3	**296**	**733**	**1029**
UTR_5	**54**	**221**	**275**
intron	**807**	**1672**	**2479**
unknown	**144**	**447**	**591**

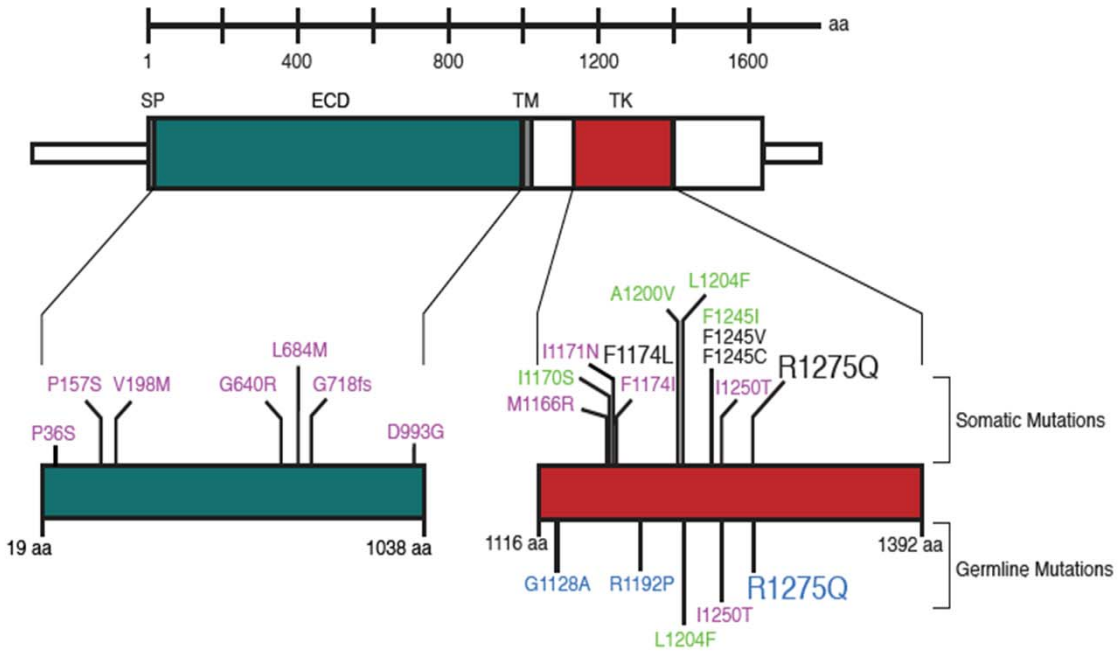
Gene	missense	splice	nonsense	frameshift	proteinDel	proteinIns	Frequenc
NOTCH1	71	3	4	7	0	0	45.21%
ALK	18	0	0	1	1	0	10.64%
CASZ1	19	0	0	0	0	0	10.11%
KIF2B	13	0	0	0	1	0	7.45%
KIF1B	11	2	0	1	0	0	7.45%
NTRK3	12	1	0	0	0	0	6.91%
GRM5	12	0	1	0	0	0	6.91%
GDF7	11	0	0	0	1	0	6.38%
CHD5	9	2	0	0	0	0	5.85%
PTPRD	8	0	0	1	0	1	5.32%
MAD1L1	9	0	0	1	0	0	5.32%
TP73	7	0	0	1	1	0	4.79%
NTRK1	7	1	0	1	0	0	4.79%
GPR153	8	0	0	1	0	0	4.79%
CAMTA1	6	0	1	2	0	0	4.79%
MMP17	6	0	0	1	0	1	4.26%
FAM55D	8	0	0	0	0	0	4.26%
PIWIL4	6	0	0	1	0	0	3.72%
P2RX7	6	0	1	0	0	0	3.72%

*Several genes (eg ALK) still with poor coverage

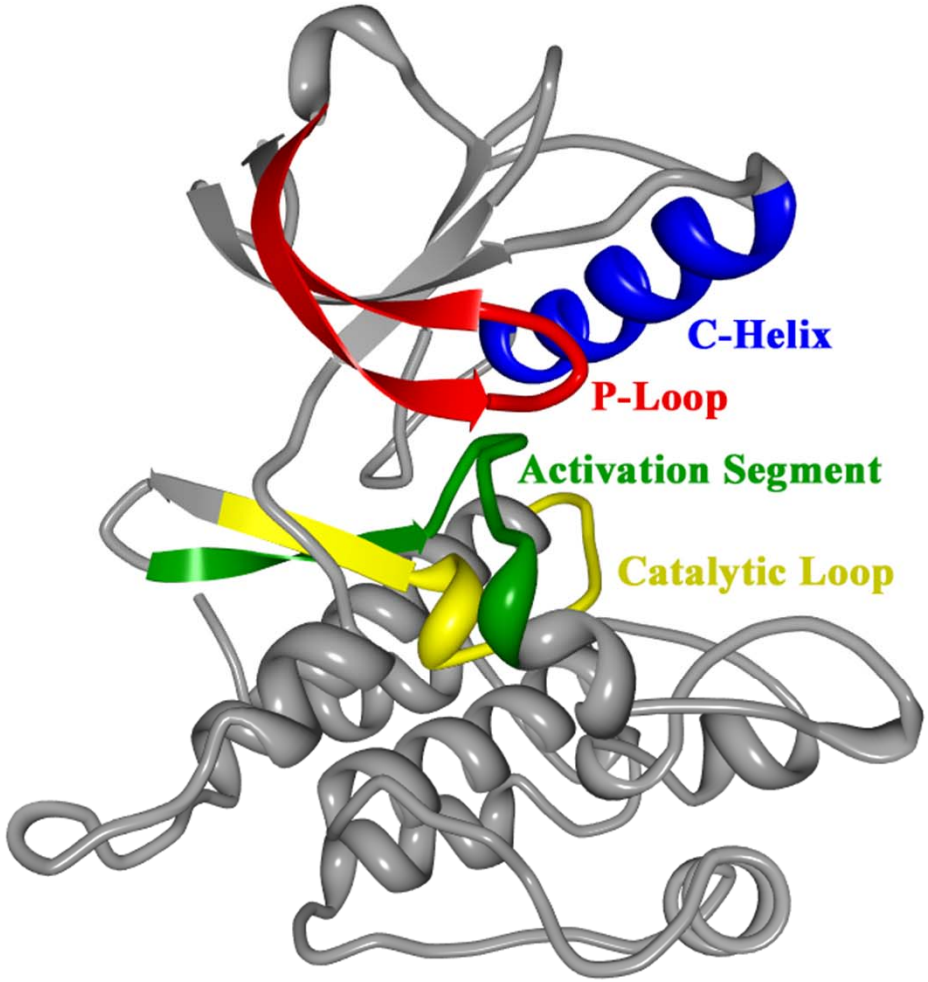
ALK is an oncogenic kinase in neuroblastoma

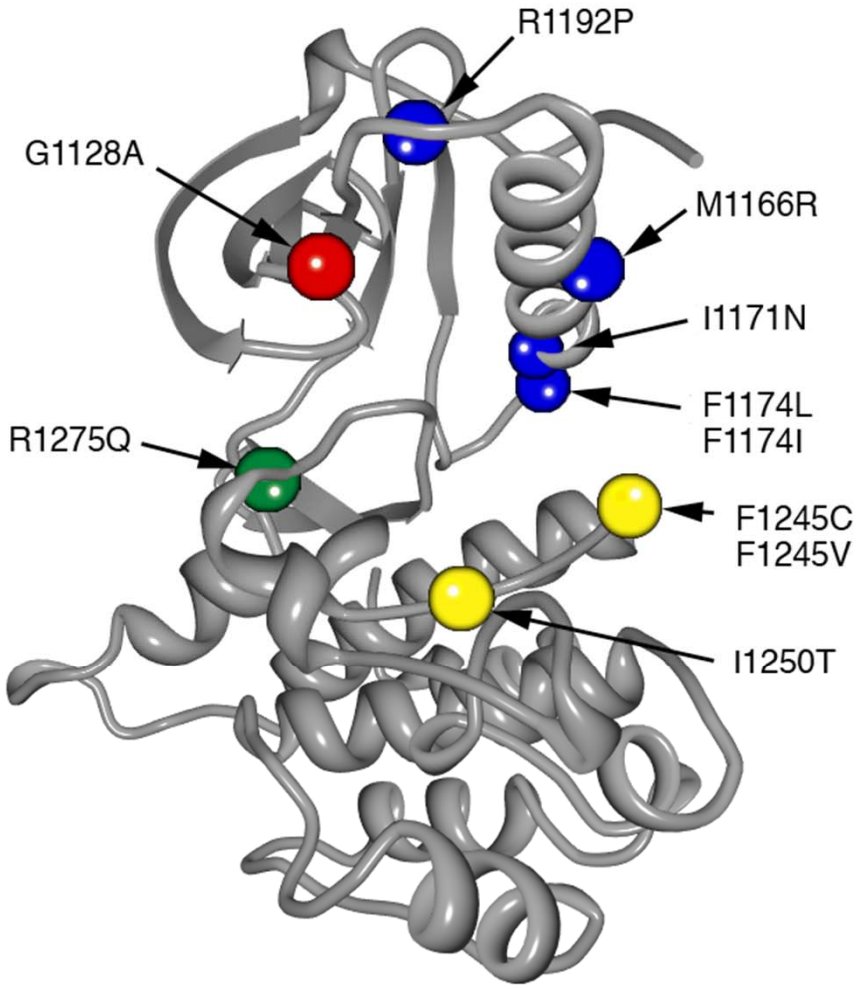
- Co-discovery of ALK as the familial neuroblastoma gene (Mosse, Nature 2008) and frequent somatic amplification and mutation (TARGET)
  - Amplification: 31/599 (5.2%)
  - Focal gain: 102/599 (17.0%)
  - Mutations in kinase domain: 43/552 (7.2%)
  - Mutations in extracellular domain: Present, frequency still be defined





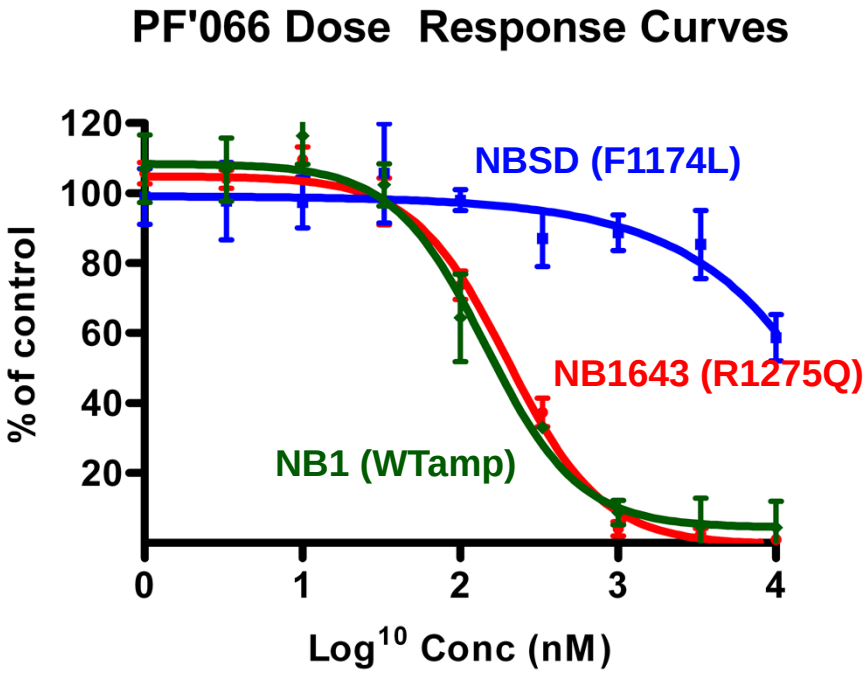
Germline and Somatic *ALK* Kinase Region Mutations Fall in Regions Shown to be Major Targets of Cancer Mutations

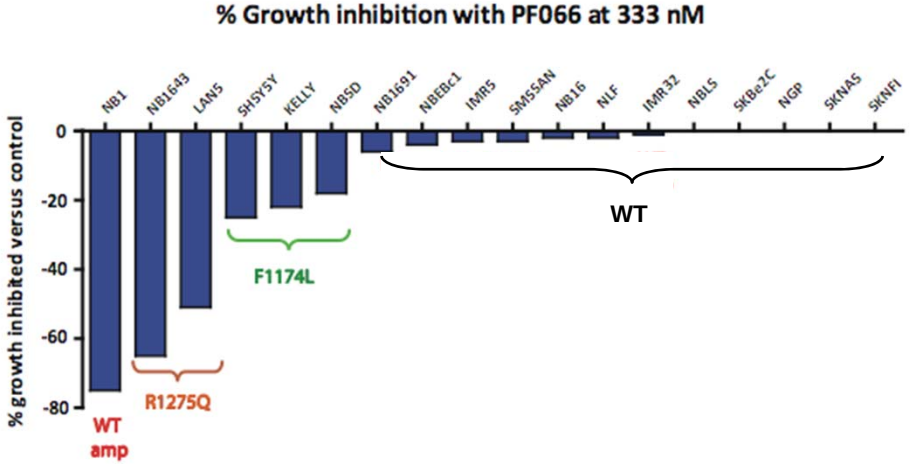




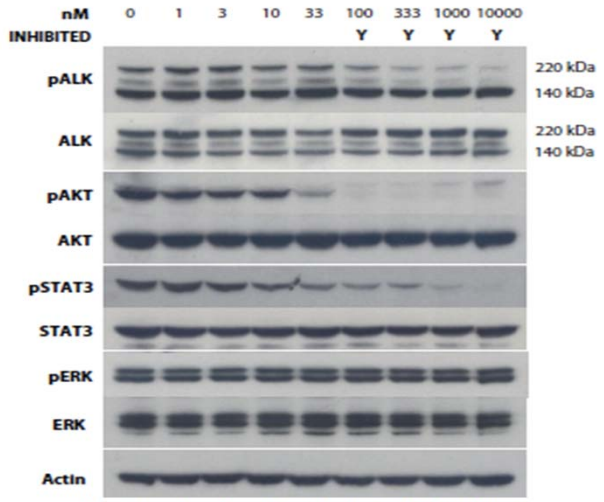
ALK is a tractable target for pharmacologic inhibition

(but sensitivity depends on mutation type)

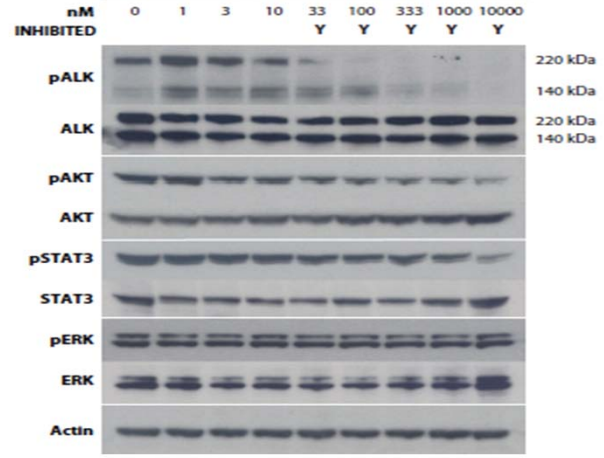




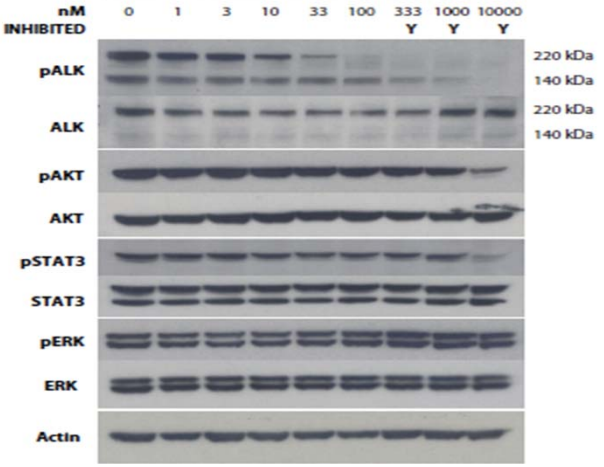
NB1 (WT amplified)



NB1643 (R1275Q)

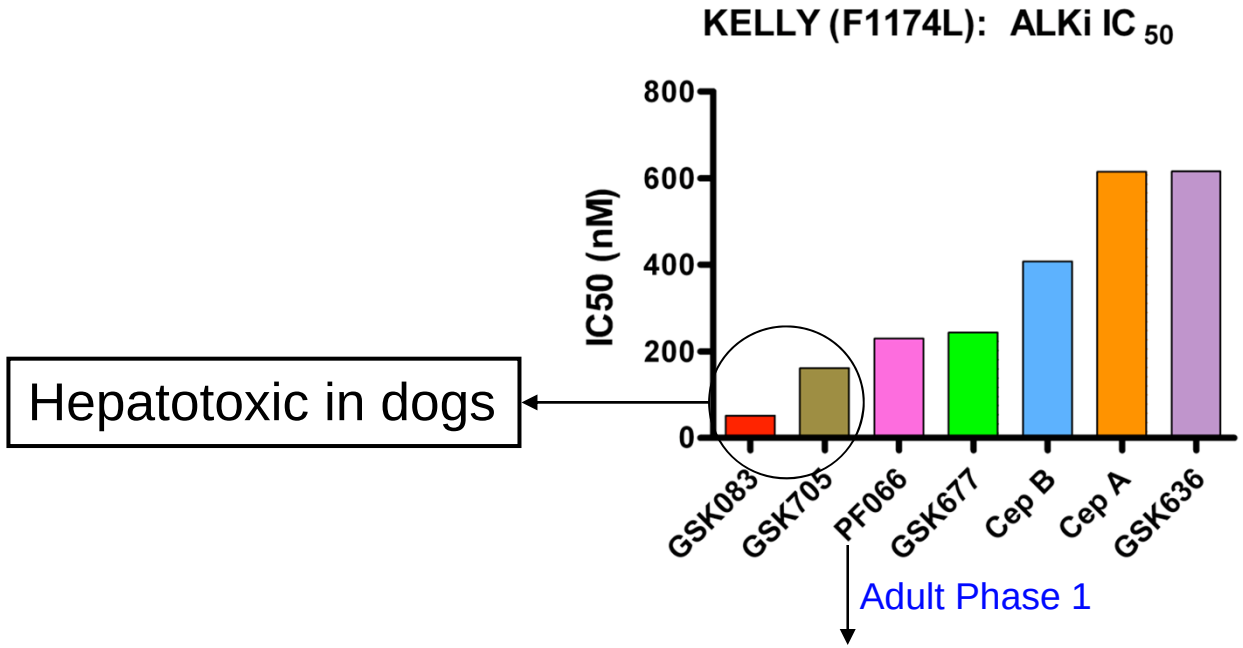


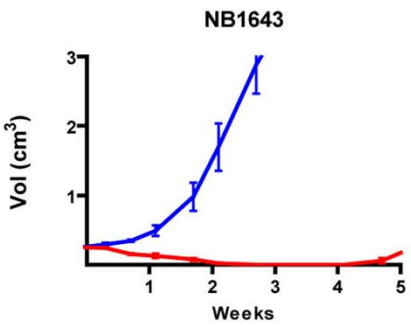
NBSD (F1174L)



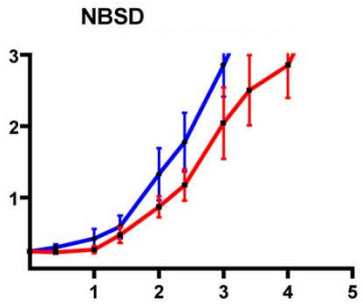
Moving ALK inhibitors to the clinic

(Which drug, which mutations?)

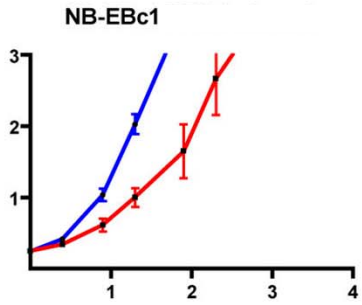




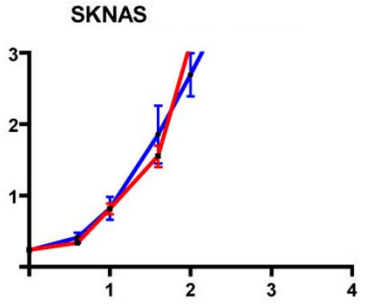
R1275Q
(Most common mutation)



F1174L
(2nd most common mutation)



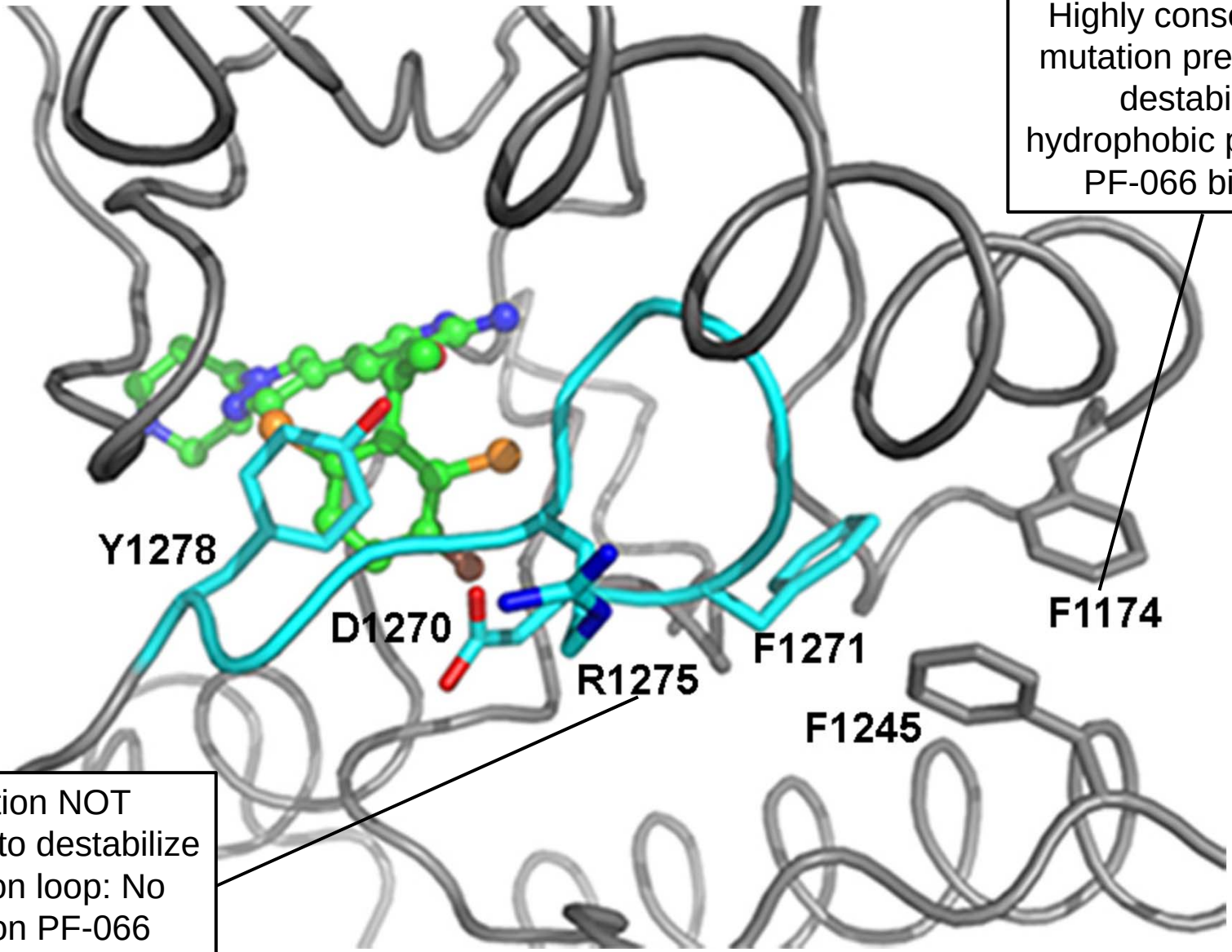
WT
(but weak pALK)



WT
(no pALK)

Getting around resistance mutations

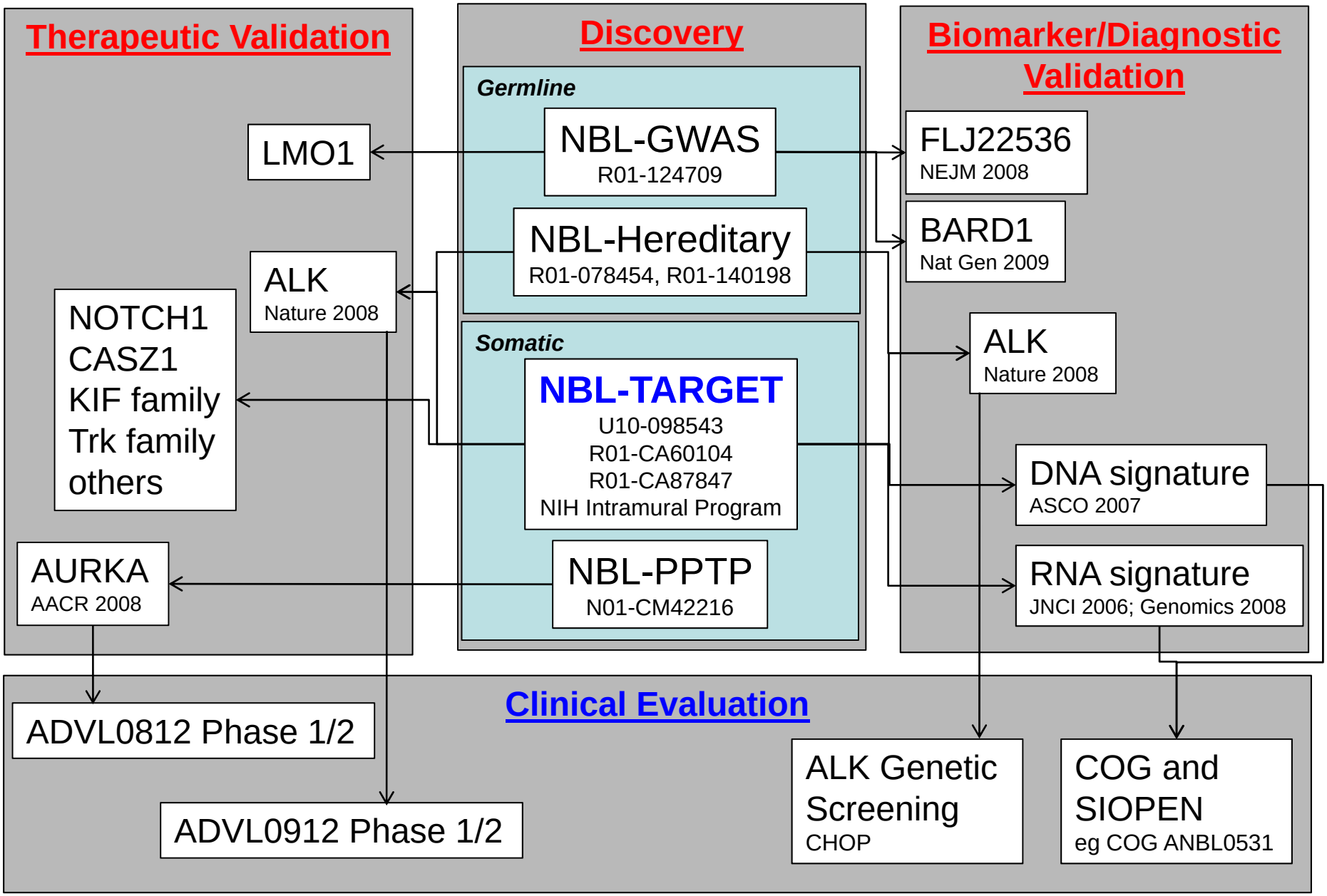
ALK homology model with PF-02341066 bound



Highly conserved F; mutation predicted to destabilize hydrophobic pocket for PF-066 binding

Mutation NOT predicted to destabilize activation loop: No effect on PF-066 binding

Discovery, Validation and Implementation



NBL-TARGET

Future goals

- Functional validation and translation of current leads ongoing
  - Focus on ALK and NOTCH1
- Consider year 1 results “proof-of-concept” with < 0.04% of genome sequenced
- Uniquely poised for a full genome sequencing effort
  - Due to size of regional aberrations, this should be done with a comprehensive epigenome profiling
    - Pilot studies on Illumina Infinium platform complete
- NBL-TARGET team has demonstrated ability to quickly validate and translate discoveries
  - Rapidly improving neuroblastoma patient care and outcome is a realistic and achievable goal of the NBL-TARGET project