LEUKEMIA2022

Rome, Hotel NH Collection - Vittorio Veneto

May 5-6, 2022

AIL President: G. Toro
Coordinators: A.M. Carella, S. Amadori

UNDER THE AUSPICES OF:

fondazione GIMEMA onlus
per la promozione e lo sviluppo della ricerca scientifica sulle malattie ematologiche. FRANCO MANDELLI

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SIE - Società Italiana di Ematologia

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University of Bologna
Department of Biomedical and Neuromotor Sciences
Section of Human Anatomy
Cellular Signalling Laboratory

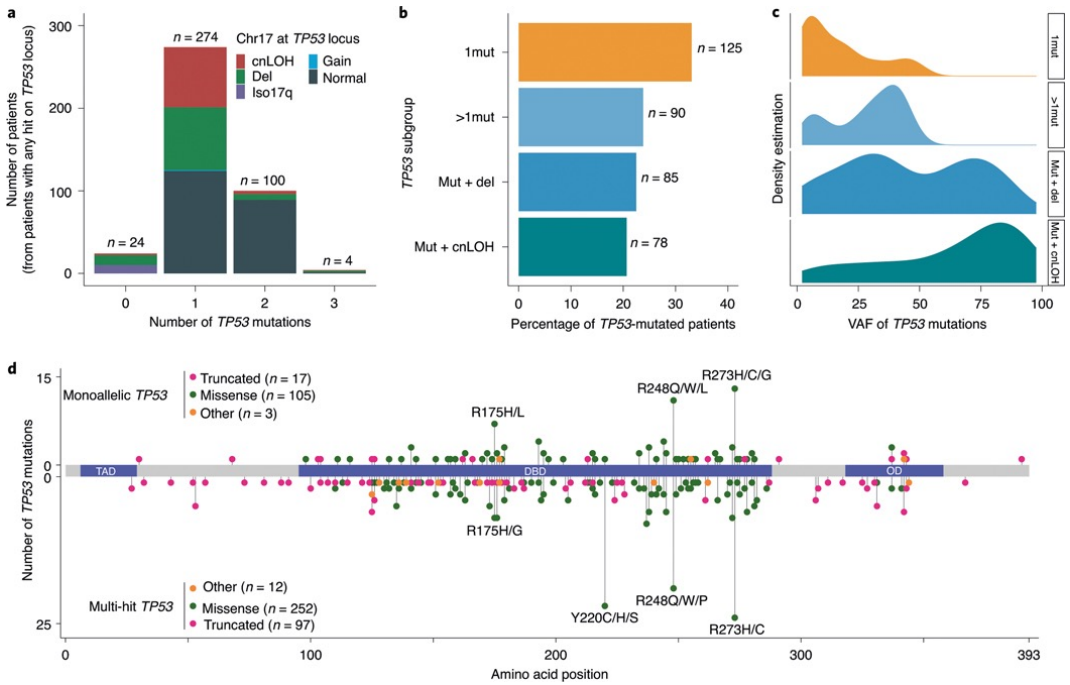
RECENT ADVANCES IN SOMATIC MUTATIONS AND ALTERED SIGNALING IN MDS CELL

Matilde Y. Follo, PhD

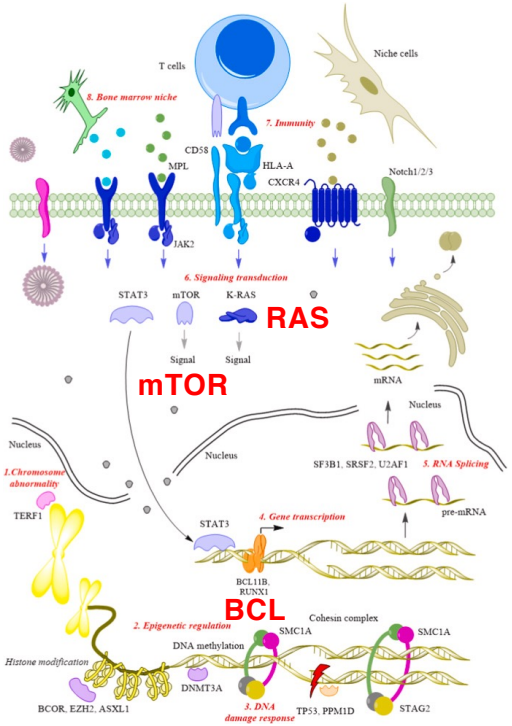
Conflict of interest disclosure

- There are no relationships to disclose

Somatic Mutations and Signalling



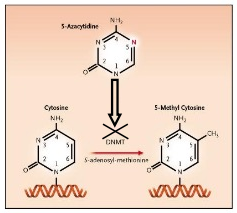
TP53 multi-hit state: *TP53* allelic state is critical for diagnostic and prognostic precision in MDS

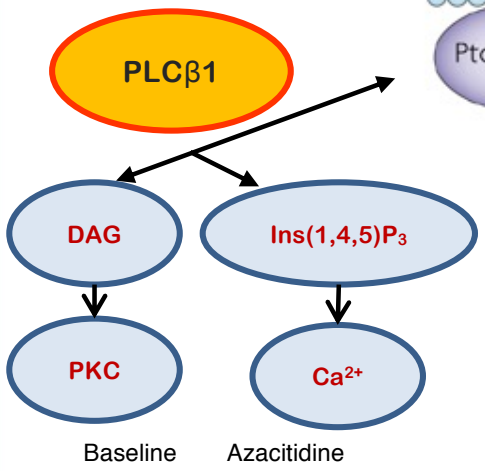


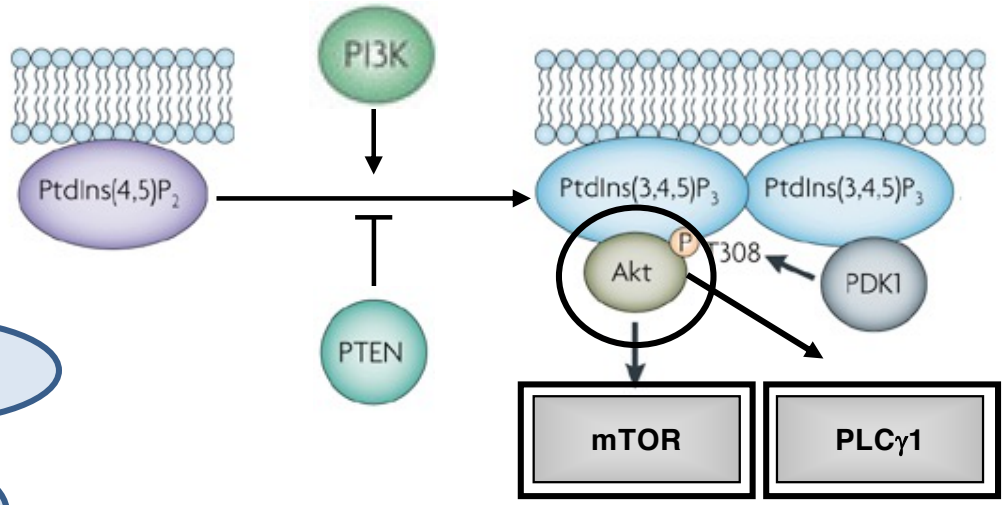
Molecular mechanisms of clonal hematopoiesis involving signaling pathways

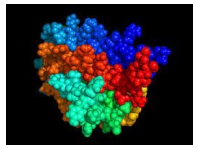
Bernard, E., … Papaemmanuil, E. *Nature Medicine*. 2020; 26(10), 1549–1556;
Ye B, et al. *Cancer Lett*. 2022 Apr 23;538:215691

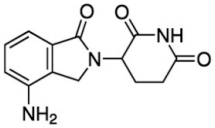
Inositide-dependent Signalling in MDS





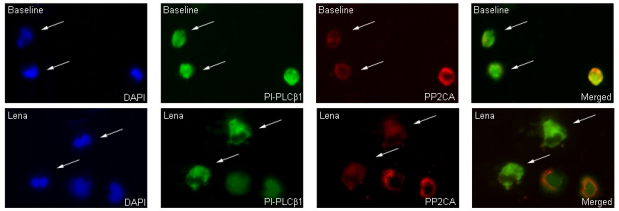




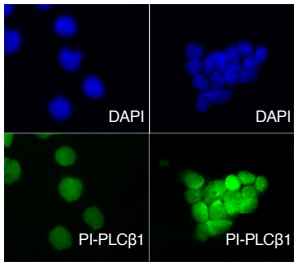


LR MDS responding to EPO:
Akt/PLCγ1 Pathway is Over-Activated

LR MDS responding to Lenalidomide:
Nuclear translocation of PKCα



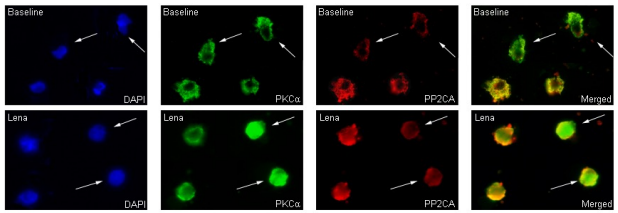
Baseline Azacitidine



HR and LR MDS at diagnosis:
Altered PI-PLCβ1 Expression

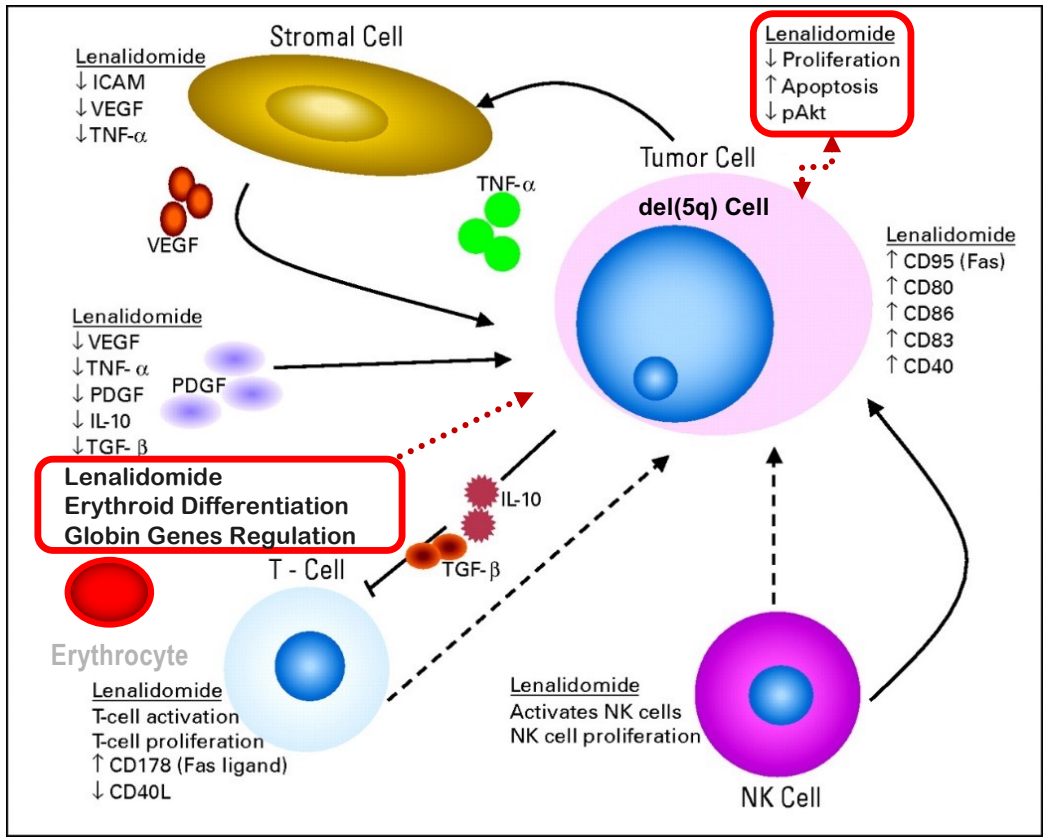
HR and LR MDS during AZA therapy:
Predictive Role of PI-PLCβ1 Expression

HR MDS at diagnosis:
Akt/mTOR Pathway is Over-Activated



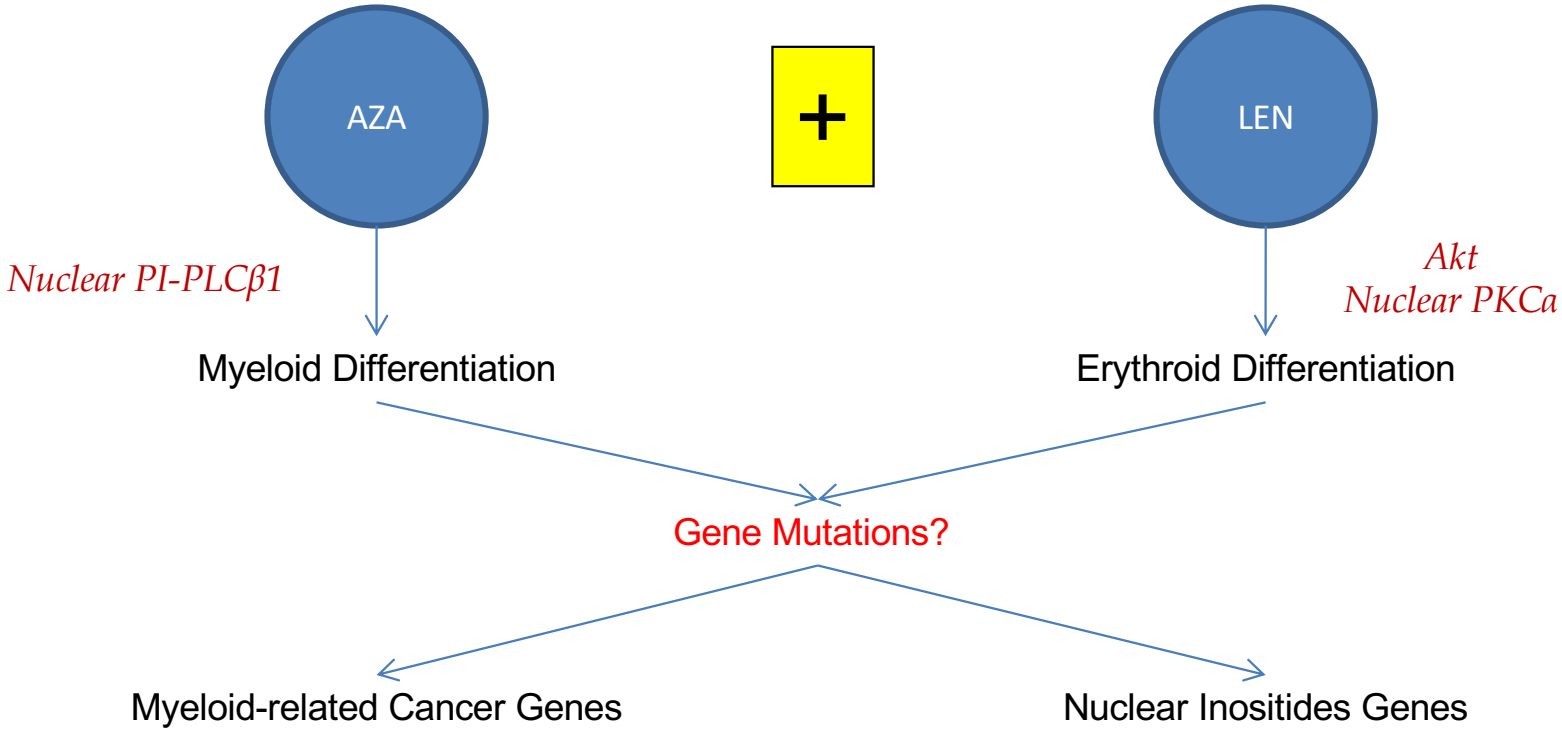
Follo MY et al. *Proc Natl Acad Sci U S A.* 2009 Sep 29;106(39):16811-6;
Follo MY et al. *Leukemia.* 2012 Dec;26(12):2474-82; Poli A et al. *FASEB J.* 2018 Feb;32(2):681-692

Lenalidomide Induces Nuclear PKCα Translocation in MDS Responder Patients

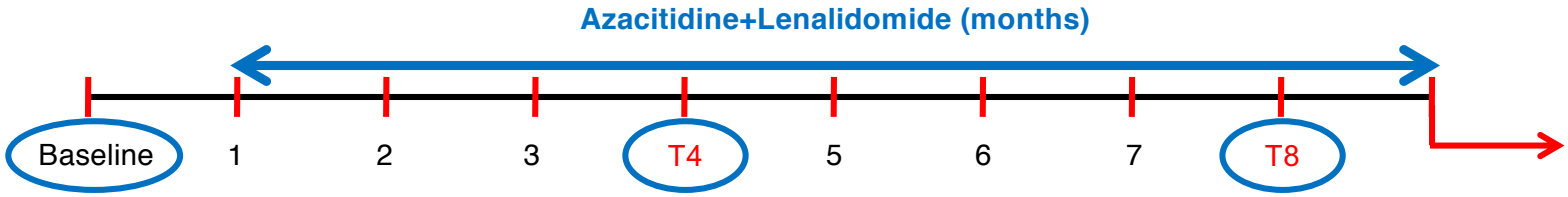


Modified from Chanan-Khan AA, et al. *J Clin Oncol.* 2008;26:1544-52; Poli A et al. *FASEB J.* 2018:681-692

Aim of the Study



Patients' Characteristics

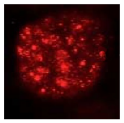


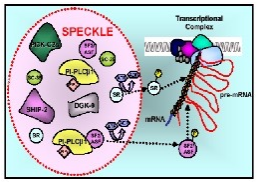
	Clinical Analysis	Molecular Analysis
No. Patients	44	30
No. Patients Evaluable for Response (≥6 cycles)	34	30
No. Patients Evaluable for Molecular Analysis	30	30
Male/Female	27/17	20/10
Median Age (Range Years)	72 (48-83)	72.5 (48-83)
WHO Classification (No. cases)	RAEB-1 (11); RAEB-2 (30); RCMD/RCMD-RS (3)	RAEB-1 (8); RAEB-2 (19); RCMD/RCMD-RS (3)
Treatment	- Azacitidine 75 mg/m2 days 1-7 - Lenalidomide 10mg days 1-21	- Azacitidine 75 mg/m2 days 1-7 - Lenalidomide 10mg days 1-21
No. Median Cycles of Treatment (Range)	8.5 (1-41)	19 (8-41)
Type of Response (no. cases)	- Responders: Complete Remission (8), marrow Complete Remission (3), marrow Complete Remission + Hematologic Improvement (6), Partial Remission (1), Hematologic Improvement (8) - Non Responders: Stable Disease (6), Disease Progression (2)	- Responders: Complete Remission (8), marrow Complete Remission (3), marrow Complete Remission + Hematologic Improvement (6), Partial Remission (1), Hematologic Improvement (8) - Non Responders: Stable Disease (4)
Median Duration of Response (Months)	10.5	12

Number of Acquired Mutations affecting Inositide-Related Genes in MDS Patients during Azacitidine and Lenalidomide Therapy (Illumina)

No. Patients Analyzed	30	Type and Duration of Response (Months)
WHO Classification *(No. cases)*	RAEB-1 (8); RAEB-2 (19) RCMD/RCMD-RS (3)	
Variant Allele Frequency (VAF): Number of Acquired Mutations during Therapy *(No. Cases, Months of Response)*	Increased VAF (5)	3 Stable Disease 2 Response (5 months)
	Decreased VAF (7) → **POSITIVE RESPONSE**	7 Complete/Partial Remission or any Hematologic Improvement (8-24 months)
	All MDS with a decreasing VAF showed a positive clinical response to Azacitidine and Lenalidomide Therapy	
Mutated Myeloid-Related Genes *(No. cases, percentage)*	ASXL1 (14, 51%) TET2 (11, 37%) RUNX1 (8, 27%)	
	SRSF2* (5, 17%) → *All Patients with SRSF2* Mutations progressed to AML*	
	No Mutations (3, 10%) → **NO AML**	

*SRSF2: aka sc-35, where nuclear PI-PLCβ1 is localized

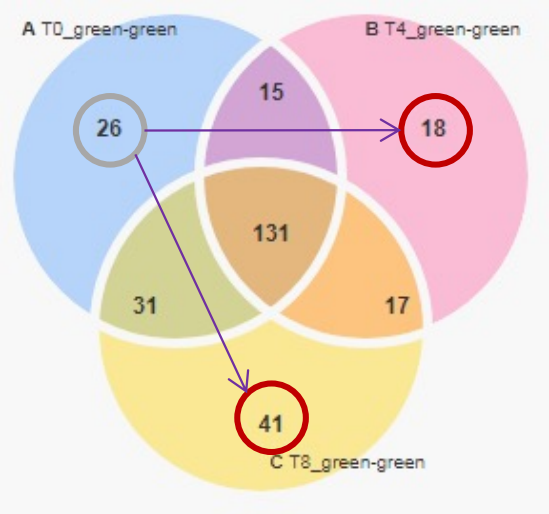




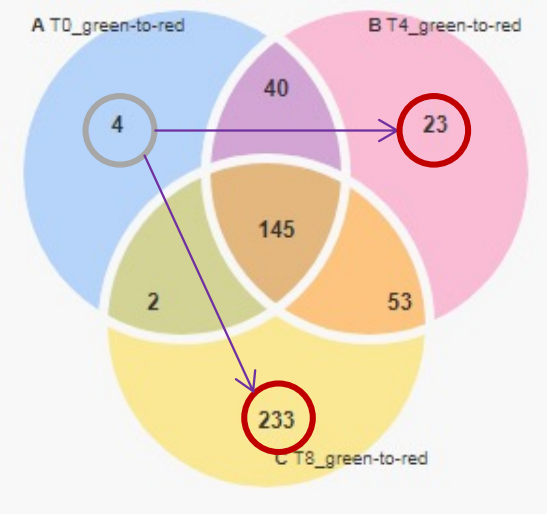
In collaboration with University of Oxford, UK

Follo MY et al. *Leukemia*. 2019 Sep;33(9):2276-2290

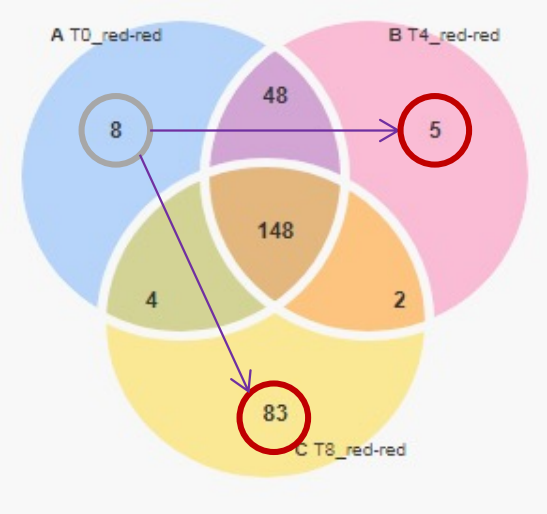
Number of Acquired Mutations affecting Inositide-Related Genes in MDS Patients during Azacitidine and Lenalidomide Therapy (Ion Torrent)



Responders (No. mutated variants)



Losing Response (No. mutated variants)



Never Responding (No. mutated variants)

All MDS patients losing response or never responding acquired a significant number of mutations during Azacitidine and Lenalidomide Therapy (T8 vs T0)

In collaboration with Dept. Cultural Heritage (UniBo), Dept. Biological Sciences (UniBo) and IRST Meldola

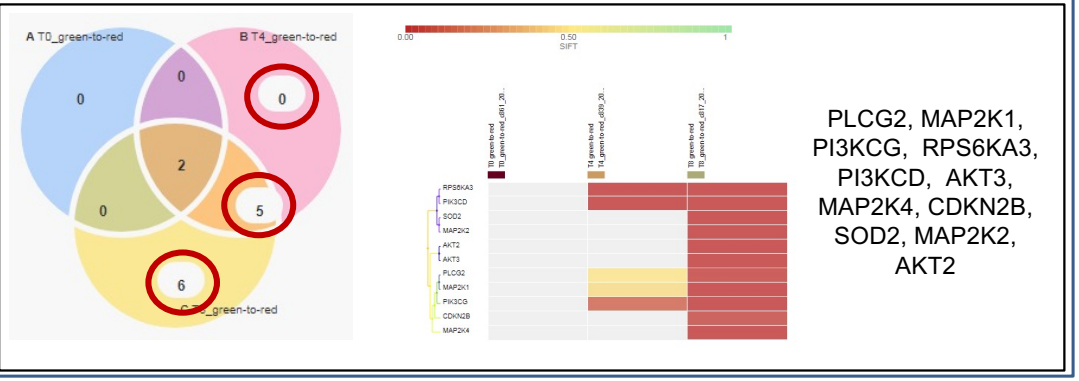
Follo MY et al. *Leukemia*. 2019 Sep;33(9):2276-2290

Number of Inositide-Related Genes with Acquired Mutations (SIFT Score) in MDS Patients Losing Response and Never Responding

Losing Response

SIFT Variant Impact:
SIFT score predicts whether an amino acid substitution affects protein function

Number of Genes with acquired mutations at T4 and T8: **11**

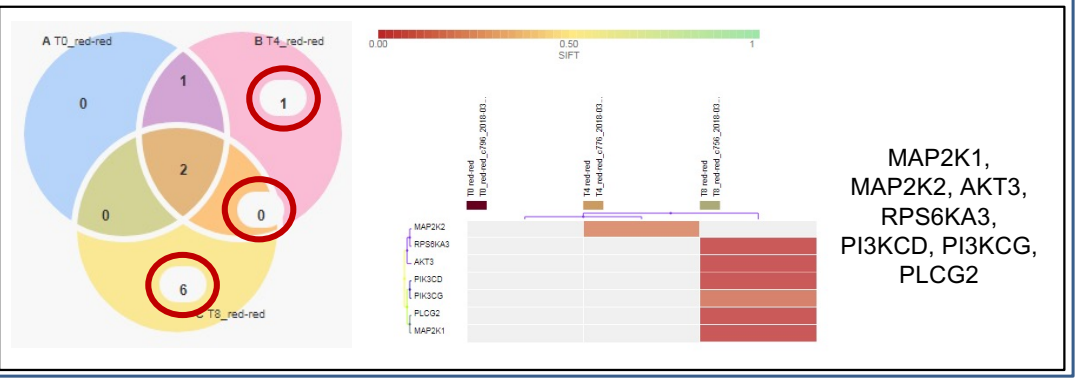


PLCG2, MAP2K1, PI3KCG, RPS6KA3, PI3KCD, AKT3, MAP2K4, CDKN2B, SOD2, MAP2K2, AKT2

Never Responding

SIFT Variant Impact:
SIFT score predicts whether an amino acid substitution affects protein function

Number of Genes with acquired mutations at T4 and T8: **7**



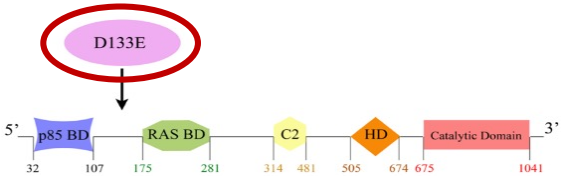
MAP2K1, MAP2K2, AKT3, RPS6KA3, PI3KCD, PI3KCG, PLCG2

Patients Losing Response or Never Responding have 6 Common Mutated Genes: MAP2K1, PI3KCD, RPS6KA3, AKT3, PI3KCG, PLCG2

Follo MY et al. *Leukemia*. 2019 Sep;33(9):2276-2290

3 Common Inositide-Specific Gene Mutations are Linked to Loss/Lack of Response

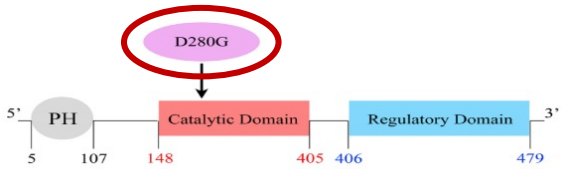
PIK3CD Proliferation/Differentiation



PIK3CD (p110δ) p.Asp133Glu D133E c.399C>G
Catalytic Subunit Delta

```
  1 MLSGPEAYVF TCINQTAEQQ ELEDEQRRLC DVQPFLPVLR LVAREGDRVK KLINSQISLL
 61 IGKGLHEFDS LQDPEVNDFR AKMCQFCEEA AARRQQLGWE AWLQYSFPLQ LEPSAQTWGP
121 GTLRLPNRAL LVNVKFEGSE ESFTFQVSTK DVPLALMACA LRKKATVFRQ PLVEQPEDYT
```

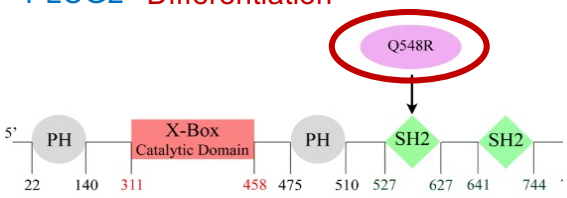
AKT3 Proliferation/Differentiation



AKT3 p.Asp280Gly D280G c.839A>G
Catalytic Domain (not "active" aa)

```
  1 MSDVTIVKEG WVQKRGEYIK NWRPRYFLLK TDGSFIGYKE KPQDVDLPYP LNNFSVAKCQ
 61 LMKTERPKPN TFIIRCLQWT TVIERTFHVD TPEEREEWTE AIQAVADRLQ RQEEERMNCS
121 PTSQIDNIGE EEMDASTTHH KRKTMNDFDY LKLLGKGTFG KVILVREKAS GKYYAMKILK
181 KEVIIAKDEV AHTLTESRVL KNTRHPFLTS LKYSFQTKDR LCFVMEYVNG GELFFHLSRE
241 RVFSEDRTRF YGAEIVSALD YLHSGKIVYR DLKLENLMLD KDGHIKITDF GLCKEGITDA
301 ATMKTFCGTP EYLAPEVLED NDYGRAVDWW GLGVVMYEMM CGRLPFYNQD HEKLFELILM
361 EDIKFPRTLS SDAKSLLSGL LIKDPNKRLG GGPDDAKEIM RHSFFSGVNW QDVYDKKLVP
421 PFKPQVTSET DTRYFDEEFT AQTITITPPE KYDEDGMDCM DNERRPHFPQ FSYSASGRE
```

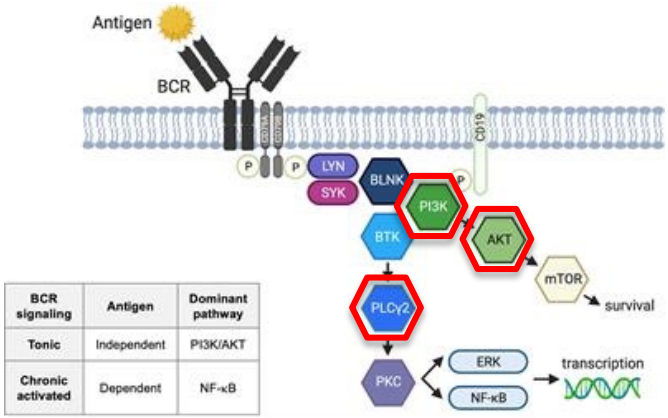
PLCG2 Differentiation



PLCG2 p.Gln548Arg Q548R c.1643A>G
N-terminal Src homology 2 (N-SH2) domain -
phosphotyrosine binding pocket (not "active" aa)

```
421 FKEVFGDLLL TKPTEASADQ LPSPSQLREK IIIKHKKLGP RGDVDVNMED KKDEHKQQGE
481 LYMWDSIDQK WTRHYCAIAD AKLSFSDDIE QTMEEEVPQD IPPTELHFGE KWFHKKVEKR
541 TSAEKLLQEY CMETGGKDGT FLVRESETFP NDYTLSFWRS GRVQHCRIRS TMEGGTLKYY
601 LTDNLTFSSI YALIQHYRET HLRCAEFELR LTDPVPNPNP HESKPWYYDS LSRGEAEDML
```

Pink aa: mutated aa from our analysis Yellow aa: "Active aa", i.e. known important aa for function

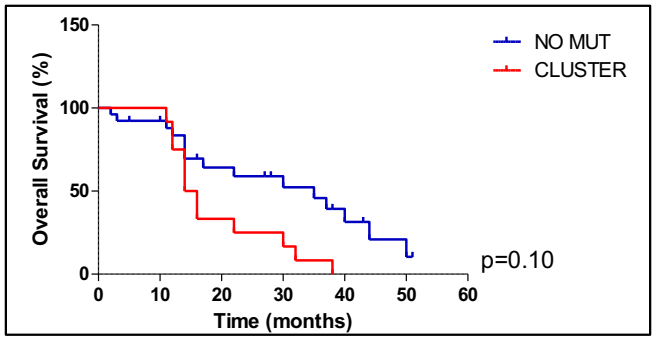


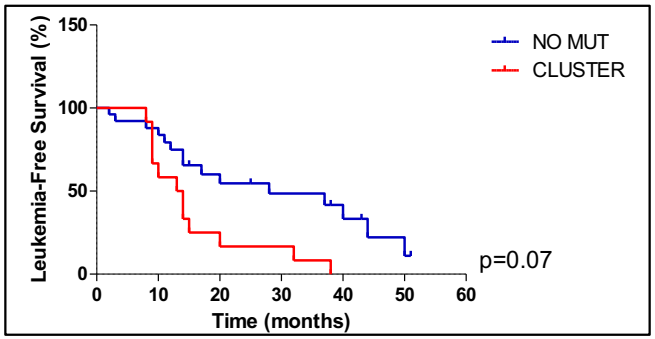
BCR signaling	Antigen	Dominant pathway
Tonic	Independent	PI3K/AKT
Chronic activated	Dependent	NF-κB

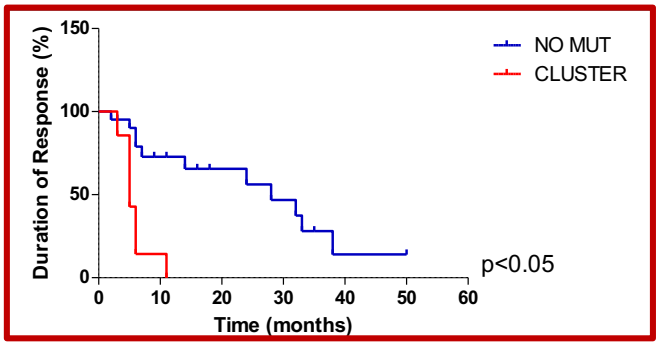
PI3K/Akt/PLCG2 activity
in immune cells, BCR signalling and CLL

Follo MY et al. *Leukemia*. 2019 Sep;33(9):2276-2290;
Ahn IE and Brown JR. Front Immunol. 2021;12:687458

Effect on Overall Survival, Leukemia-free Survival, Duration of Response







The presence of all our 3 Mutations (PI3KCD, AKT3, PLCG2) is significantly associated with a shorter duration of response to Azacitidine and Lenalidomide Therapy

Follo MY et al. *Leukemia*. 2019 Sep;33(9):2276-2290

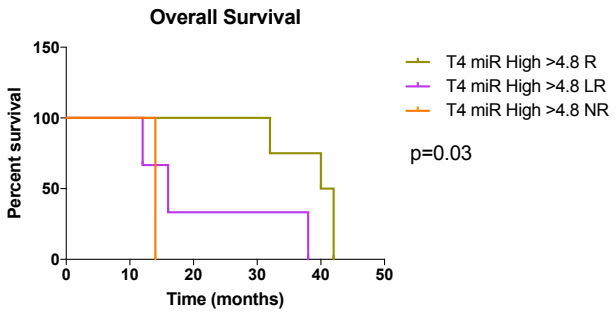
Conclusions and Perspectives

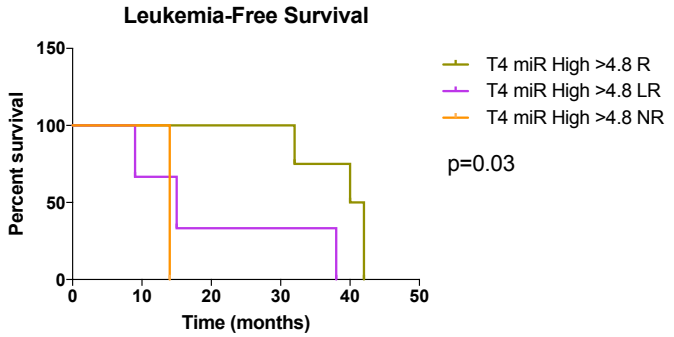
- AzaLen Therapy can induce a favourable response in MDS patients
- AzaLen Therapy change the VAFs and mutation profiling of inositide-specific genes, with a cluster of 3 mutated genes associated with loss/lack of response (PI3KCD, AKT3, PLCG2)

↓

Specific mutagenesis of mutated genes to determine their in vitro effect

miRNA Targets: miR-192-5p





miR-192-5p increased gene expression at T4 correlates with better OS and LFS in Responders, hinting at a prognostic relevance that may improve patients' stratification

Acknowledgments, Collaborations and Funding

Cellular Signalling Laboratory
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KBRI and UNIST, South Korea
Pann-Ghill Suh, PhD, DVM

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UNIVERSITÀ DEGLI STUDI FIRENZE

Prof.ssa Valeria Santini

MIUR PRIN

ALMAIDEA ALMA MATER STUDIORUM UNIVERSITÀ DI BOLOGNA

FONDAZIONE DEL MONTE DI BOLOGNA E RAVENNA