VICHEM CHEMIE
THE KINASE INHIBITOR COMPANY

SEMMELWEIS UNIVERSITY,
DEPARTMENT OF MEDICAL CHEMISTRY
MTA–SE PATHOBIOCHEMISTRY RESEARCH GROUP

IN VITRO COMBINATION THERAPY BASED ON DRIVER GENES

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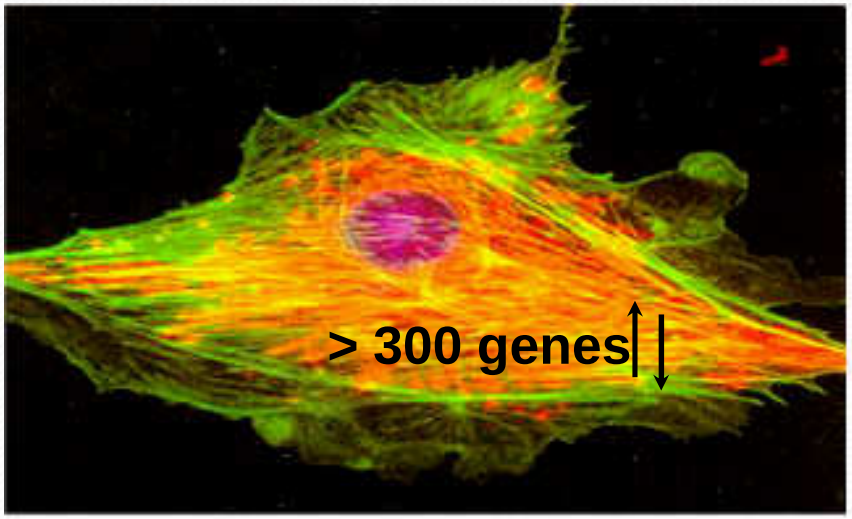
SIGNAL TRANSDUCTION THERAPY IN CANCER

- Identification of oncogenic signaling – several 100 genes are overexpressed in tumor cells vs. normal cells, therefore levels of thousands of proteins in the protein-protein interaction network change.
- **Which is the cause and which is the causative?** - Which are the **Driver or Passanger** genes? – Molecular Diagnostics are needed
- **Acquired resistance** against most of the cancer drugs because of feed-back loops, cancer stem cells, transporters, selection of resistant mutants or complete driver pathway switch.
- **Breakthrough in cancer reserch:** Identifying the Driver genes and inhibiting Driver related cancer pathways - inducing apoptosis and killing all the tumor cells



Driver genes and survival signals (rate limiting steps)
– Inhibition of survival signals – mostly kinases with kinase inhibitors

Sharma K, … Keri G, … ***NATURE METHODS*** 6:(10) pp. 741-744. (2009)
Petak I, … Keri G ***NATURE REVIEWS DRUG DISCOVERY*** 9:(7) pp. 523-535. (2010)
Szokol B, … Keri G ***ACS MEDICINAL CHEMISTRY LETTERS*** 5:(4) pp. 298-303. (2014)
Torka R, … Keri G, … ***NEOPLASIA*** 16:(4) pp. 301-318. (2014)

BREAKTHROUGH IN CANCER RESEARCH – THEORETICAL BASIS: VOGELSTEIN MODEL

- Vogelstein's model: **12 cancer pathway** that regulates cell fate, cell proliferation and cell death (Vogelstein et al, Science Vol 339, 2013)
- Cancer pathways are coordinated by **cancer driver genes**
- Driver gene identification basis: **COSMIC database** v61 containing 405,271 mutations (current update v72 contains 3,158,657 and expanding)
- Considering the **mutational patterns** Vogelstein set up the 20/20 score criteria for the identification (20% tumor suppressor or oncogene score)
- Based on COSMIC v61 he identified **138 driver genes** (**64** driver oncogenes and **74** driver tumor supressor genes)

Our goal is to provide multiple inhibition strategies for all cancer pathways via inhibiting the cancer driver genes' effect, by pathway analysis based protein targeting for personalized therapy and to overcome resistance

CANCER IS GENOMIC DISEASE – DRIVER MUTATIONS IN NUMBERS

Bert Vogelstein:
AACR 2010 Meeting
Plenary Session
Science 2013 March

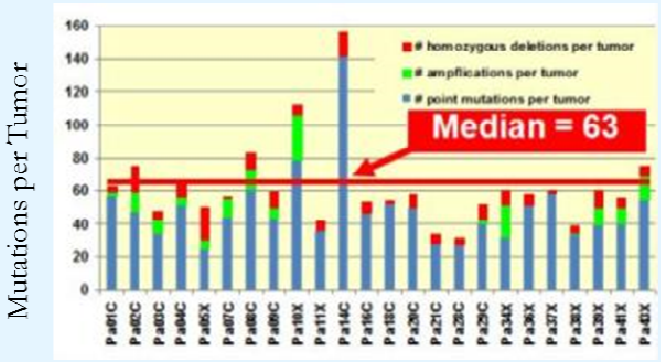
Review of Literature/Databases

- 3284 tumor – 18306 mutated genes
- 405.271intragenic somatic mutations

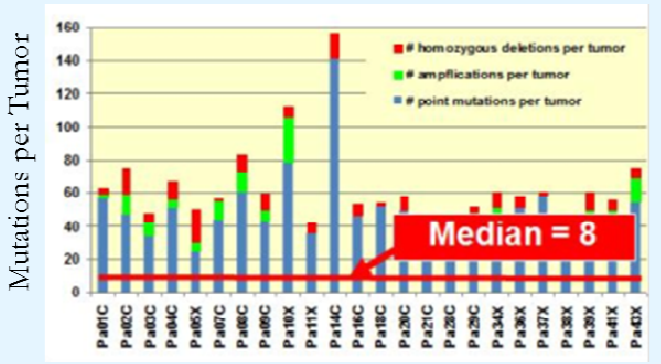
Driver Genes:

- 64 oncogenes (>20% of recorded mutations in the gene are at recurrent positions and are missense)
- 74 tumor suppressor genes (>20% of the mutations in the gene are inactivating)
- Average 33 to 66 genes display subtle somatic mutations .
- 2-8 Driver genes / tumor

Genetic Alterations in Pancreatic Cancer



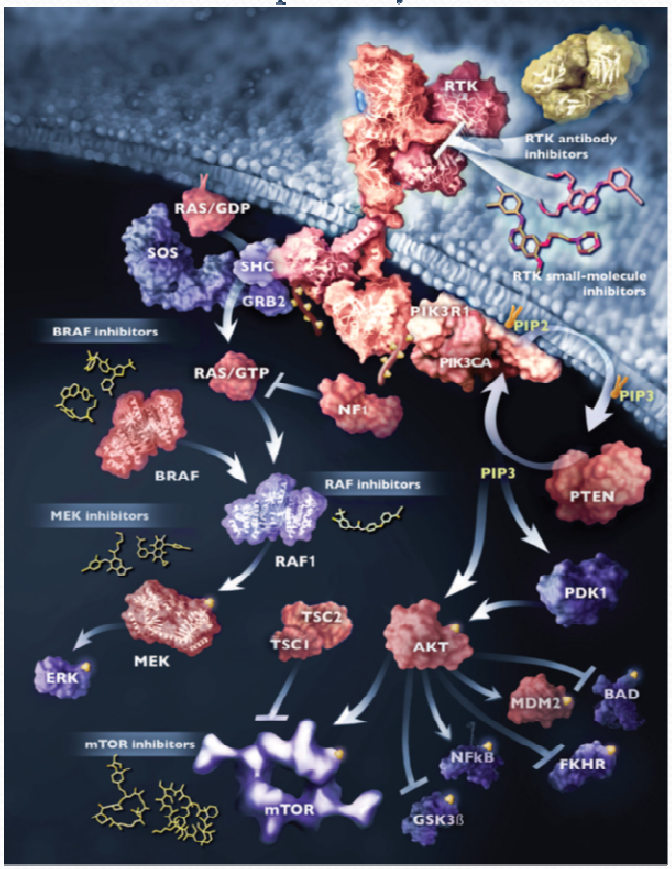
Driver Gene Alterations in Pancreatic Cancer



SIGNAL TRANSDUCTION PATHWAYS

Cancer cell signaling pathways
and the cellular processes they regulate

Mutation affected pathways



Vogelstein B, et al. Science 2013, 393: 1546-1558.

DRIVERHIT LIBRARY™ AND CONCEPT

- **Theoretical basis, know-hows and tools** developed:
  - Theory: the Vogelstein model and it's extensions
  - Database, with references for drivers, driver targets and compounds
  - Target and compound finder methods
  - Dedicated compound library
- **Proof of concept studies** on
  - Solid tumor types
  - Multiple myeloma cell lines and surviving cultures
- **DriverHit projects by drivers and driver targets**

DRIVERHIT CONCEPT FOR PERSONALIZED GENOMIC MEDICINE

Mission: To provide personalized targeted treatment combinations for cancer patients to treat the disease, overcome primary and secondary resistance due to multiple driver cancer genes present in the patients' tumor: Personalized Drug Discovery Service

Vision: Cancer can be cured with the right combination of cancer pathway targeted therapies.

Our goal is to create a revolutionary scientific and business model to fight cancer through integration of molecular information services, molecular diagnostics services and drug discovery.

Our concept is based on the identification and the blockage of the effect of the cancer driver genes and the related cancer pathways for killing all the cancer cells.

PERSONALIZED GENOMIC MEDICINE

- Identify the driver (2-8) and the concomitant mutations from the patient samples even in 0.1% of the cells (resistance causing or cancer stem cells due to tumor heterogeneity)

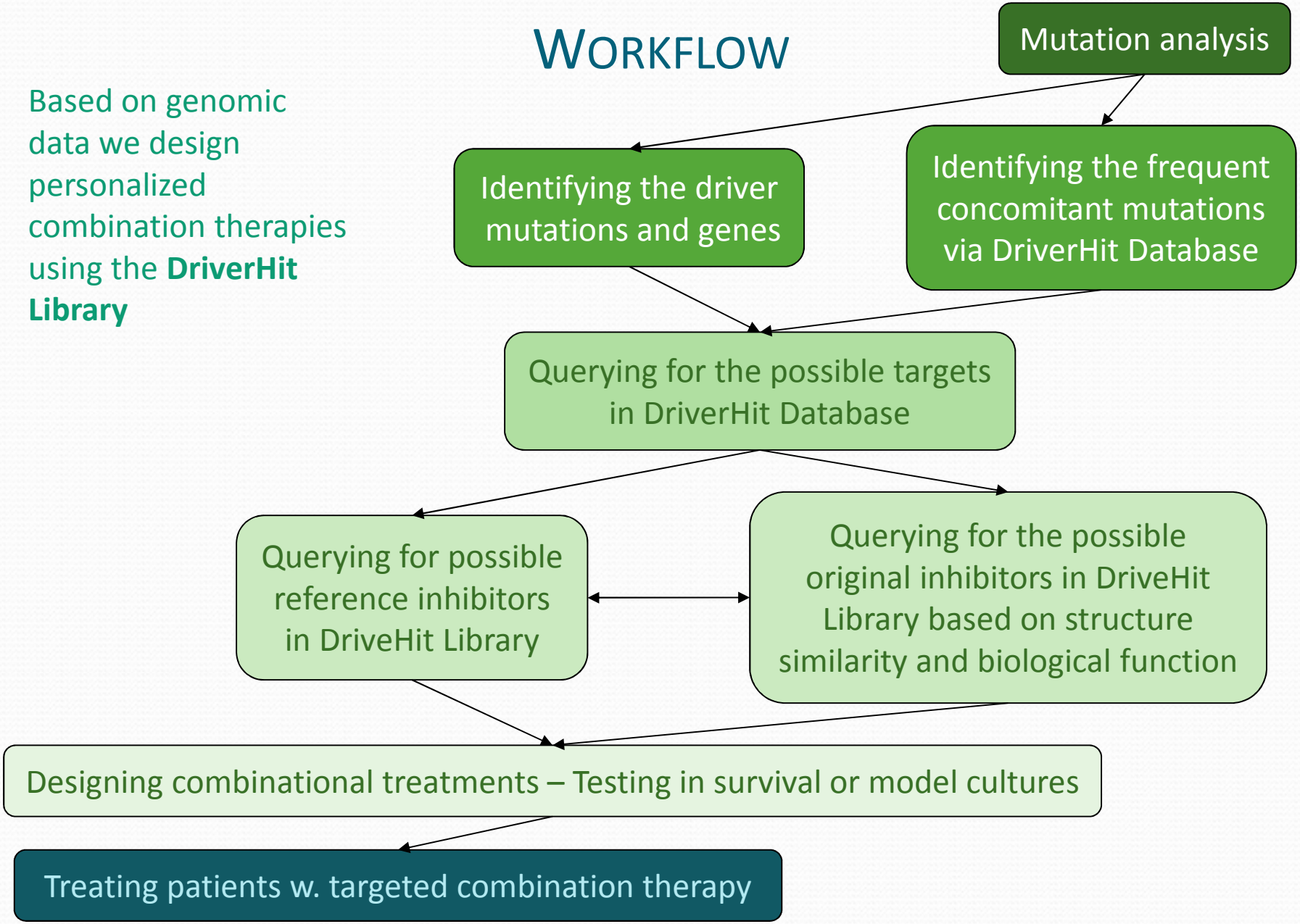
- Identify the cancer pathways (activated by these drivers) which are essential for the tumor cells including the small populations of resistance causing cells

- Recommend the proper combination of drugs against the identified driver related cancer pathways to kill all the cancer cells (even those which are in a very small portion)

- Test the selected targeted drug combination *in vitro* on the survival culture of the patient's tumor cells isolated from the tumor biopsy or survival culture of circulating tumor cells

WORKFLOW

Based on genomic data we design personalized combination therapies using the **DriverHit Library**

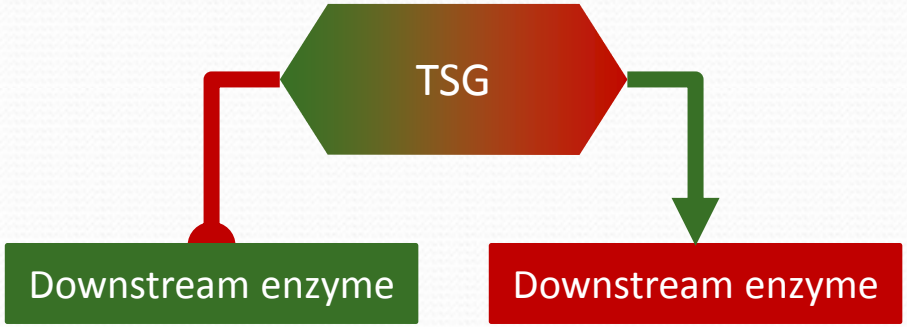


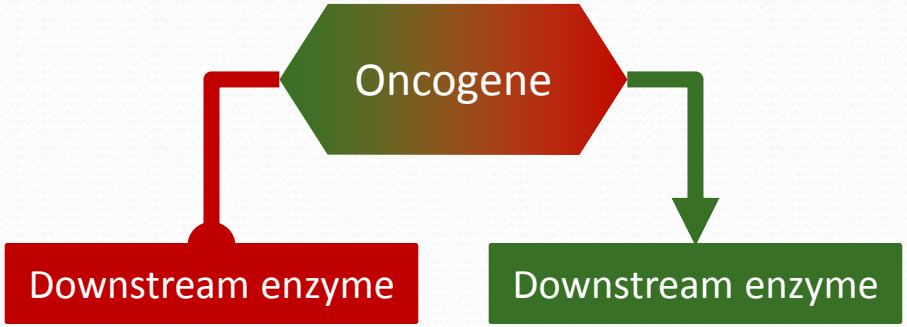
TARGET IDENTIFICATION METHOD I.

In the first step the Algorithm distinguishes by the basic nature of the given driver gene (TSG/oncogene)

Tumor suppressors: loss of function mutated TSGs are not targets however the downstream elements of the cancer pathway could be targets.

Oncogenes: if it has enzyme activity which can be inhibited by small molecule inhibitors the first method is to target the oncogene **directly**.





We target the **proteins which are naturally inhibited/suppressed** by the TSG, but are activated by the mutant TSG.

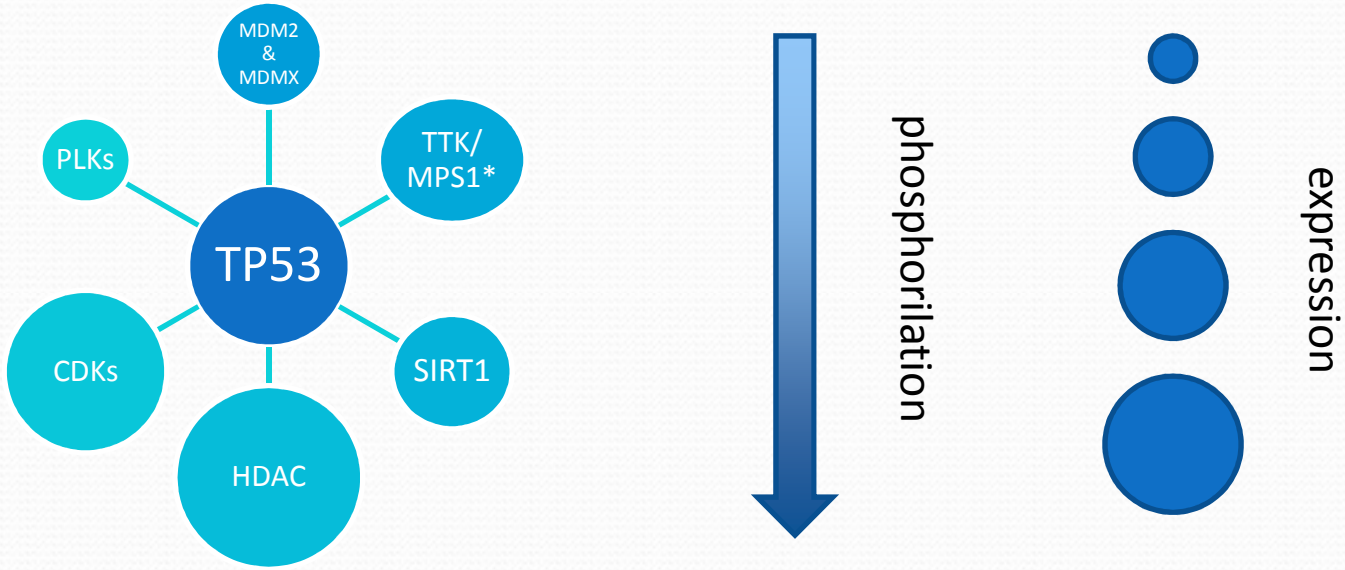
If the driver oncogene cannot be a target, the **nearest downstream stimulated enzyme** should be the target in the cancer pathway.

Target identification: analysing concomitant mutations, epigenetic regulators for transcriptome, RNAseq, phosphoproteomics for pathway analysis

Target validation: in survival cultures and with CRISPR/Cas9 technology

TP53, A TSG DRIVER – WHAT TO TARGET?

We can reduce the fraction of proteins to target by simply querying the DriverHit Database and the Algorythm comes up with potential drugs and their combinations which are likely to be effective at the same time.



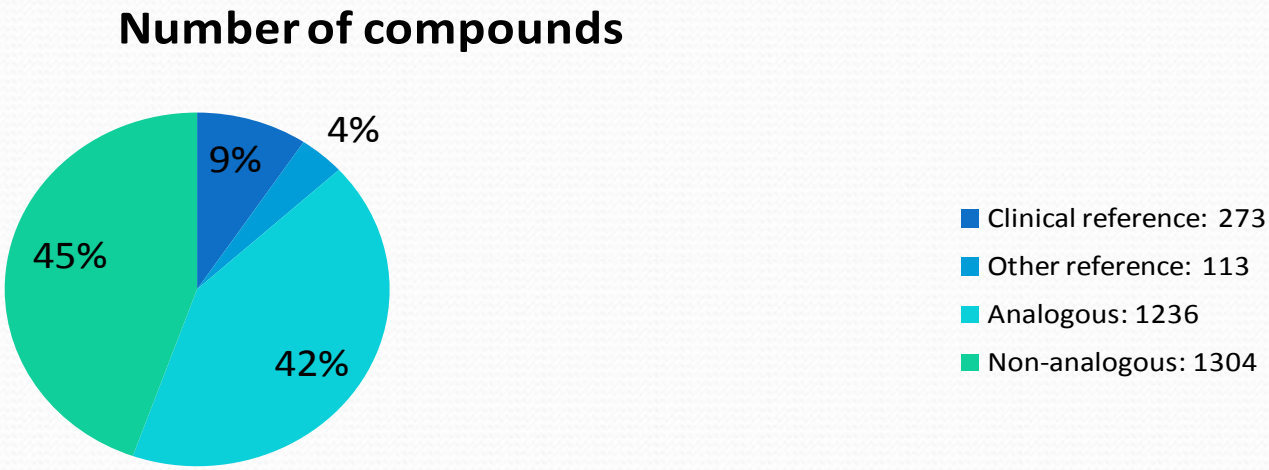
Steps of narrowing down the potential targets and target combination set:

1. based on a search for each TSG in DriverHit Database we can narrow down the given set of proteins and protein pairs to target
2. ranking the targets by expression level give us a sharper picture whether the proteins selected in the previous step are expressed
3. ranking the overexpressed targets by the actual phosphorylated portion gives us information about the expressed proteins that are really active in the given cancer

Győrffy B, ... Kéri G, Orfi L, ... Santarpia L. Mol Oncol. 2014 May;8(3):508-19.

DRIVERHIT LIBRARY™ – CONTENTS IN NUMBERS

- The library currently contains **2926** compounds including **more than 700** patentable or Vichem patented compounds for drug development
- Overall number of the reference compounds is **386**
- With these 386 reference compounds we can **target 114 driver genes** out of **138** and by this we are able to **block all of the cancer pathways** at numerous points



COMBINATION THERAPY ON MYELOMA AND COLON CELL LINES

Sample	• Validated sequencing of the 138 driver genes (KeatsLab)
Specific drivers	• Identification of the cell line/tissue specific drivers in the given sample
Targets	• Identification of the potential targets (Pathway Analyzer Algorythm)
Drugs	• Choosing small molecular weight drugs which inhibit the aimed targets (DriverHit Library™)
Mono-therapy	• Determining the inhibitory potential of the driver targeted monotherapies (in-house measurements)
Combina-tion therapy	• Based on the monotherapy results using the Pathway Analyzer Algorithm we determine the potential targets and drugs to combine
Synergism analysis	• Based on the combination therapy results we determine the best drug combination(s) which are the most synergistic and most effective ones at the same time

DHL PROOF OF CONCEPT STUDIES ON CANCER CELL LINES

- Proof of concept study on colon (HT29 and HCT-116), lung (A549) and multiple myeloma (RPMI-8226, U266, LP1) cancer cell lines

- 3 levels of study:
  - monotherapy
  - combination therapy with 50-50% compound ratio
  - ratio optimized combination therapy

DHL PROOF OF CONCEPT STUDIES ON SOLID TUMOR CELL LINES

- Selecting compounds based on the DriverHit concept proved to be the **most efficient* compared to the control designs in HT29 and MCF7**, namely 1. bests in monotherapy among each other 2. random pairing

- On all cell lines involved **the 10 best combinations out of 28 were always based on the DriverHit concept**, although only two Drivers were targeted

- The **rate of improvement**** for the best combinations constructed using DriverHit is ranging from **400-fold to 45000-fold improvement compared to the monotherapies**.

* Most efficient: mean of all improvement constructed upon a given concept was compared
** formula of the rate of improvement value for compound A and B:
(IC50 in monotherapy of A/IC50 in combination)+(IC50 in monotherapy of B/IC50 in combination)

DRIVER-TARGET RELATIONSHIPS IN MYELOMA CELL LINES

The myeloma cell lines' mutations were obtained from the Multiple Myeloma Cell Line Charachterization Project performed by Jonathan Keats and lab.

RPMI8226		U266		LP-1	
Drivers	Target(s)	Drivers	Target(s)	Drivers	Target(s)
ARID1A	AKT, PI3K, mTOR, AR	ARID1A	AKT, PI3K, mTOR, AR	CASP8	DNMT1, DNMT3A, HDAC
MAP3K1	MEK, AR	MAP3K1	MEK, AR	MAP3K1	MEK, AR
ARID1B	AKT, PI3K, mTOR, AR	ARID1B	AKT, PI3K, mTOR, AR	ARID1B	AKT, MTOR, AR
MLL3	HDAC, proteasome	MLL3	HDAC, proteasome	MLL3	HDAC, proteasome
NOTCH1	NOTCH s	NOTCH1	NOTCH s	CDKN2A	CDKs
KRAS	KRAS, Ftase, MEK	KRAS	KRAS, Ftase, MEK	TP53	HDAC, AURKA, MYC, CDKs, BCL2
TP53	CDKs, MYC, HDAC, AURKA, BCL2, PLK1	TP53	CDKs, MYC, HDAC, AURKA, BCL2, Topoisomerase II		
JAK3	JAKs, AR	JAK3	JAKs , AR		

DRUG COMBINATIONS AGAINST MULTIPLE MYELOMA I. RPMI-8226

- we designed 75 combinations in total by combining 16 small molecule inhibitors
- 50 were bicomponent, 25 consisted of 3 different compounds
- 48 combinations out of 75 designed ones, 64%. were synergistic
- 26 two-membered combinations were synergistic out of 50 (52%), while in the case of three-membered combinations 22 out of the 25 three-membered combinations were synergistic (88%)

GX15-070
- Driver: TP53
- Targets: BCL2
- IC95 in monotherapy: 0.08191 µM

Trametinib
- Driver: MAP3K1, KRAS
- Targets: MEK1/2
- IC95 in monotherapy: 30+ µM

Dinaciclib
- Driver: TP53
- Target: farnesyl transferase
- IC95 in monotherapy: 0.0181 µM

95% cell killing at 28 nM (0.028 µM) when utilizing 1:1:1 ratio

CI95= 0.003060

DRUG COMBINATIONS AGAINST MULTIPLE MYELOMA II. U266

- we designed 102 combinations
- 74 were bicomponent, 28 consisted of 3 different compounds
- 25 combinations out of 102 were synergistic (25%)
- 15 two-membered and 10 three-membered combinations were synergistic

GX15-070
- Driver: TP53
- Targets: BCL2
- IC95 in monotherapy: 51.6491 µM

Dp44mT
- Driver: TP53
- Targets: DNMT1
- IC95 in monotherapy: 0.0031 µM

BI2536
- Driver: TP53
- Target: PLK
- IC95 in monotherapy: 30+ µM

95% cell killing at 56.52 nM (0.05652 µM) when utilizing 1:1:1 ratio

CI95= 0.018070

DRUG COMBINATIONS AGAINST MULTIPLE MYELOMA III. LP1

- we designed 86 combinations
- 65 were bicomponent, 21 consisted of 3 different compounds
- 48 combinations out of 86 were synergistic (56%)
- 31 two-membered combinations were synergistic out of 65 (48%) while 17 three-membered combinations were synergistic out of 21 (81%).

Dinaciclib
- Driver: TP53, CDKN2A
- Targets: CDKs
- IC95 in monotherapy: 0.0181 μM

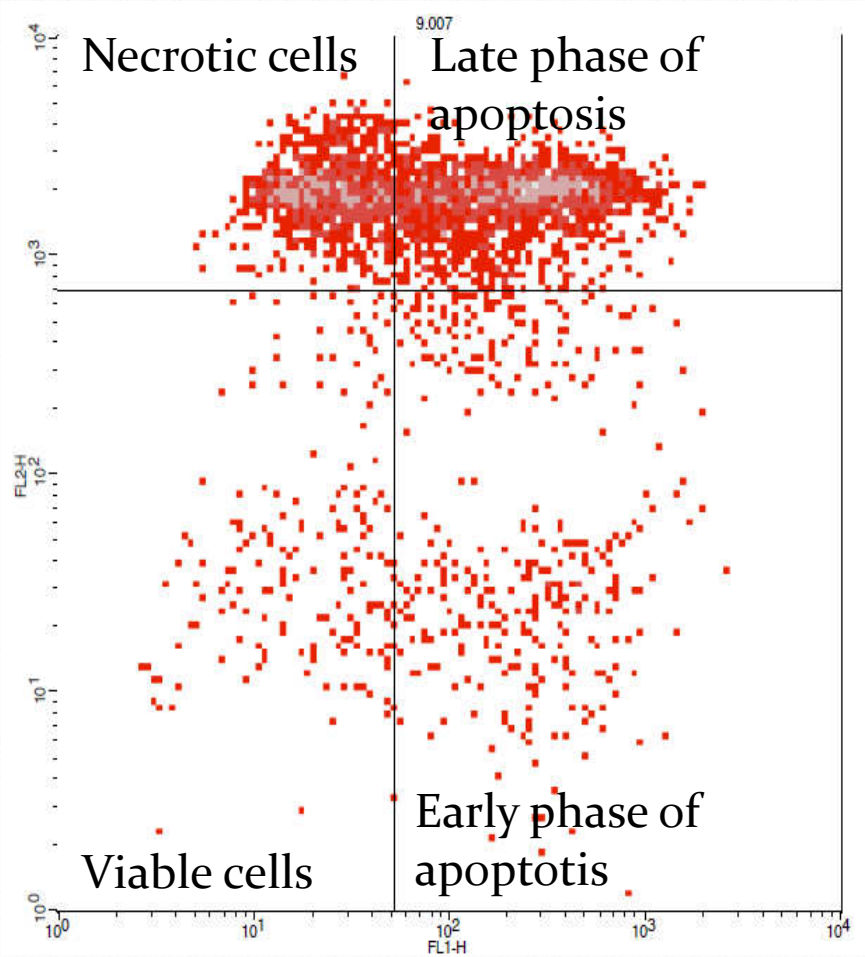
GSK2126458
- Driver: ARID1B
- Targets: PI3K-mTOR
- IC95 in monotherapy: 0.02213 μM

CUDC907
- Driver: TP53, MLL3
- Target: HDAC
- IC95 in monotherapy: 0.09210 μM

95% cell killing at 1.68 nM (0.00168 μM) when utilizing 1:1:1 ratio

CI95= 0.06235

LP1 FACS MEASUREMENTS – BEST COMBINATION



Dinaciclib + GSK2126458 + CUDC907

0.1 µM

48 hours

PI+ Annexin (FL2 FL1 channel)

5.23% viable cells

DRUG COMBINATIONS AGAINST COLON CANCER I. – HT29

- drivers in HT29: APC, BRAF, MLL3, PIK3CA, SMAD4, TP53
- measuerements and their evaluation are in progress, we designed 86 combinations so far
- The colon cell lines' mutations were obtained from COSMIC database

Hesperadin
- Driver: APC
- Targets: CHEKs
- IC95 in monotherapy: 3.03643 μM

GSK2126458
- Driver: PIK3CA
- Targets: PI3K-mTOR
- IC95 in monotherapy: 45.8848 μM

Vorinostat
- Driver: TP53, MLL3
- Target: HDAC
- IC95 in monotherapy: 4.11453 μM

95% cell killing:
565.94 nM (0.565 μM)
when utilizing 1:1:1 ratio

CI95=0.11209

DRUG COMBINATIONS AGAINST COLON CANCER II. – HCT-116

- we designed 77 combinations
- out of the 77 HCT-116 combinations 37 had two and 40 had three drug members
- 43 combinations out of 77 were synergistic (56%)
- 16 two-membered combinations were synergistic out of 37 (43%) while 27 three-membered combinations were synergistic out of 40 (68%)

KRAS Inhibitor 12
- Driver: KRAS
- Targets: KRAS
- IC95 in monotherapy: 109.536 µM

Danusertib
- Driver: TP53
- Targets: AURKA
- IC95 in monotherapy: 5.17668 µM

GX15-070
- Driver: TP53
- Target: BCL2
- IC95 in monotherapy: 0.16097 µM

95% cell killing at 122 nM when utilizing 1:1:1 ratio

CI95= 0.261750

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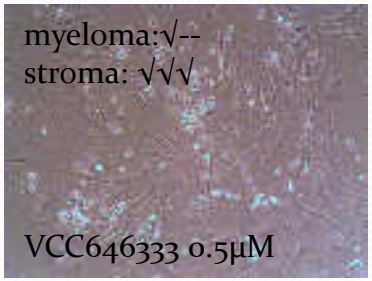
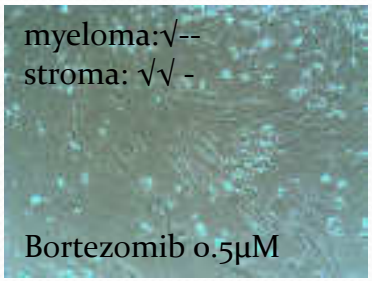
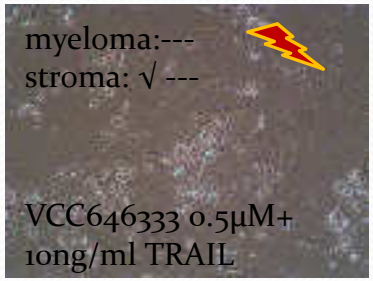
COMBINATION THERAPIES WITH TRAIL ON A PATIENT DERIVED SURVIVING CULTURE

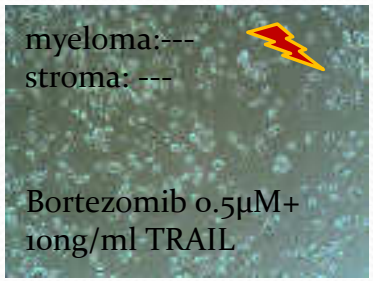
Myeloma & stroma co-culture

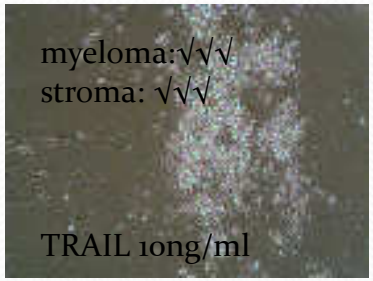




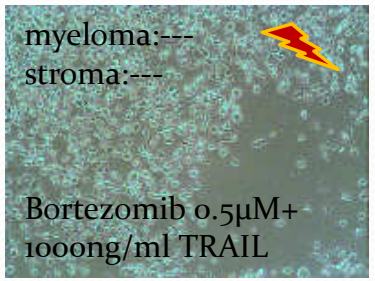
The CDK inhibitor only affects myeloma cells and Bortezomib affects mostly myeloma cells

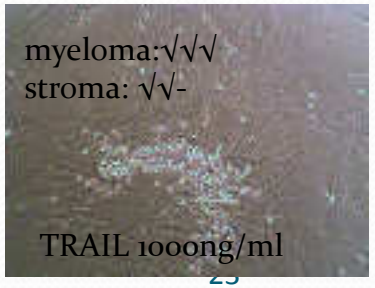






TRAIL sensitizes myeloma and stroma cells for the CDK inhibitor and Bortezomib although doesn't have an affect as a single agent





TRAIL is synergistic with the CDK inhibitor and Bortezomib in a concentration dependent manner but only have mild effect on stroma cells alone

- The surviving culture was derived from a patient of Szent László Hospital
- The cultures are myeloma an stroma cell containing models
- The number of √s correlates with cell viability
- VCC646333 is a CDK inhibitor developed by Vichem Ltd.

SUMMARY

- Vichem Ltd. has developed the **DriverHit Concept**, which is a **target and drug identification system based on the driver gene mutation pattern** in a given cell line or patient.

- Vichem has set up a proprietary compound library, the **DriverHit Library®** which contains **inhibitors against drivers and driver targets**

- Based on the Concept we also determined that which driver targets should be targeted together in combinations. We performed **combination therapies** based on the Concept using compounds from the DriverHit Library **on multiple cell lines and patient derived surviving cultures**

- We found that DriverHit compounds show **synergisms** and these synergisms **allows us to reach total cell killing in very low concentrations**

ACKNOWLEDGEMENT

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THE END

The sea is in the drop and the drop is in the sea,
Serving Life (and Chemistry) is our destiny…
(Life is Love… and Chemistry)