Deregulation in Translation and Small Molecules

Prabhat Arya
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www.signmod.org

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Dr. Reddy's Inst of Life Sciences, University of Hyderabad Campus

Prepared for Drug Discovery and Development Workshop
March 15-April 4, 2021

My Journey: Academic to Entrepreneurial Path

Academic Tenure in India (2009-2018)

Past Graduate Students

Madhu Aeluri
Srinivas Chamakuri
Ravikumar Jimmidi
Shiva Krishna Reddy
Bhanudas Dasari
Srinivas Jogula
Saidulu Konda
Mahender Khatravath
Naveen Kumar
Jagan Gaddam

DR. REDDY'S INSTITUTE OF LIFE SCIENCES

Our Biotech Journey (2018-2020)

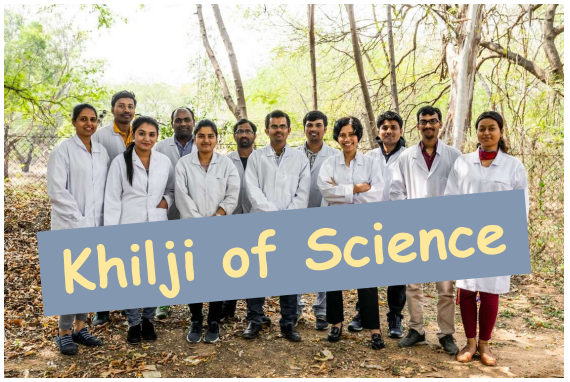
SignMod TRANSCELL Oncologics

Cell signaling/Biology:

Raveendra Babu
Vamshi Krishna
Anusha Kolusu
Samarpita Tarafder
Manjushri

NGS:

Madhu Mohan

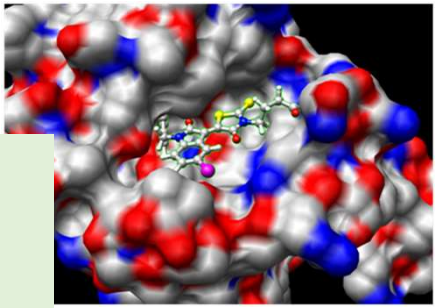


Chem toolbox/med chem:

Jagan Gaddam
Naveen Kumar
Mahender Khatravath
Anand
Neha Kardam

Classical Way of Going Forward…

Defining the target (could be enzymes or isolated protein(s)



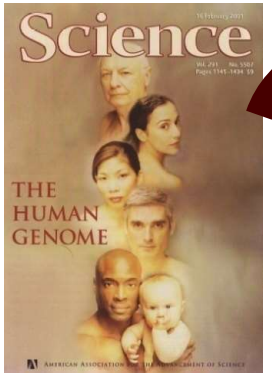
Structural information on the target – finding the pocket

Structural guided chem /med chem

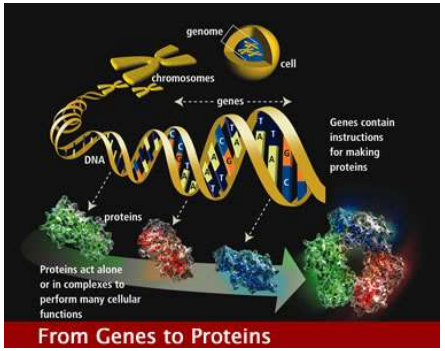
Lipinski's Rule of 5

Pre-genomic Era

Post-genomic Beginning



From genes to pathways:



tough journey

- Complex, multiple protein-protein interactions
- Dynamic and temporal processes!
- Regulation (normal) and de-regulation (disease)

Paradigm Shift

nature

NEWS & VIEWS FEATURE

2005

A new grammar for drug discovery

Mark C. Fishman and Jeffery A. Porter

To realize the potential of the genome for identifying candidate drugs we must move beyond individual genes and proteins. The signalling pathways in cells provide the right level for such analyses.

…Our current understanding of molecular pathways is insufficient as a platform for effective pharmaceutical discovery…

…Several biotechnology companies have focused on the known elements of a few key pathways to target them with new medicines. But for the genome to be translated into medicines with any reliability and regularity, far more work needs to be done. Defining the role of pathways in complex diseases will undoubtedly take many years…

Elias A. Zerhouni is President of Global R&D, Sanofi, 75008 Paris, France, and former Director of the U.S. National Institutes of Health.

Citation:
E. A. Zerhouni, Turning the Titanic. *Sci. Transl. Med.* **6**, 221ed2 (2014).

2014

DRUG DISCOVERY

Turning the Titanic

AT THE END OF THE 20TH CENTURY, BIG PHARMA AND ITS CUSTOMERS EXPERIENCED heady days. Translation of medicines such as cholesterol-lowering agents, HIV protease inhibitors, and the first molecularly targeted cancer drugs improved lives and enriched the pharmaceutical industry. The recipe for success appeared obvious: Tweeze apart biological pathways in model systems, and pinpoint molecular targets likely to be pivotal in a disease process. Use this information to develop high-throughput assays to screen for drug candidates. Test promising lead compounds in animal models of disease, and optimize the winners by using medicinal chemistry. Demonstrate safety and efficacy in clinical trials in order to satisfy the approval requirements of regulators, and deploy in the marketplace to benefit patients.

Expensive? Yes. But for a time, the formula was successful often enough to make medical and financial sense. More recently, costly failures in late-stage clinical trials have stalled the Titanic, and these leaks in the translational pipeline have produced a biomedical innovation gap: Most newly marketed drugs are close relatives of already approved, rather than first-in-class, entities (*1*).

Underestimated the complexity of human biology!

Protein-Protein Interactions Arena!

Science **2003**, *300*, 445-452 **REVIEW**

Assembly of Cell Regulatory Systems Through Protein Interaction Domains

Tony Pawson[1,2]* and Piers Nash[1]

The sequencing of complete genomes provides a list that includes the proteins responsible for cellular regulation....

However, this does not immediately reveal what these proteins do, nor how they are assembled into the molecular machines and functional networks that control cellular behavior.

CHEMICAL REVIEWS

2014

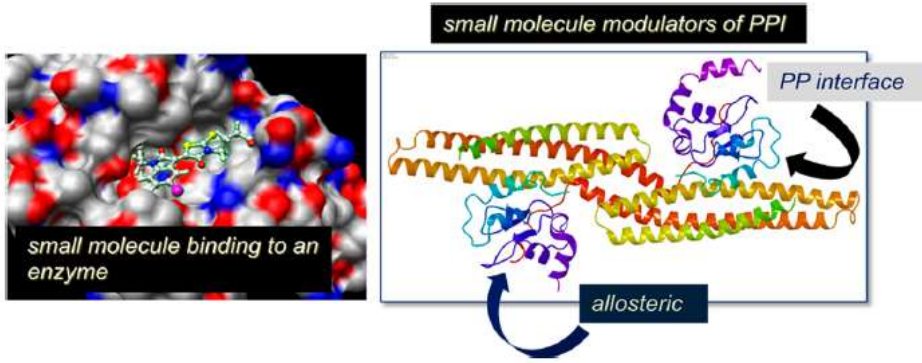
Review

pubs.acs.org/CR

Small Molecule Modulators of Protein−Protein Interactions: Selected Case Studies

Madhu Aeluri,[†] Srinivas Chamakuri,[†] Bhanudas Dasari,[†] Shiva Krishna Reddy Guduru,[†] Ravikumar Jimmidi,[†] Srinivas Jogula,[†] and Prabhat Arya*

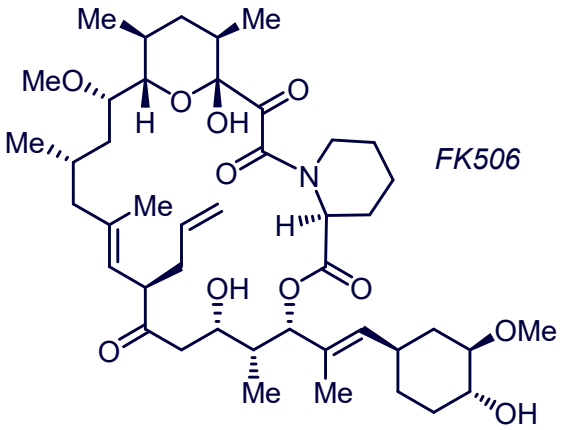
Dr. Reddy's Institute of Life Sciences (DRILS), University of Hyderabad Campus Gachibowli, Hyderabad 500046, India



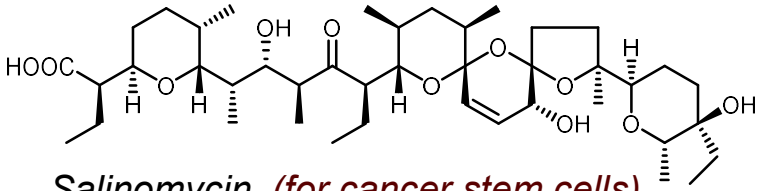
- Map large surface area
- Shallow surface
- Combination of several weak interactions
- Extended hydrophobic interactions
- Possible *hot spots*

Finding Molecules as Effective Modulators of Pathways?

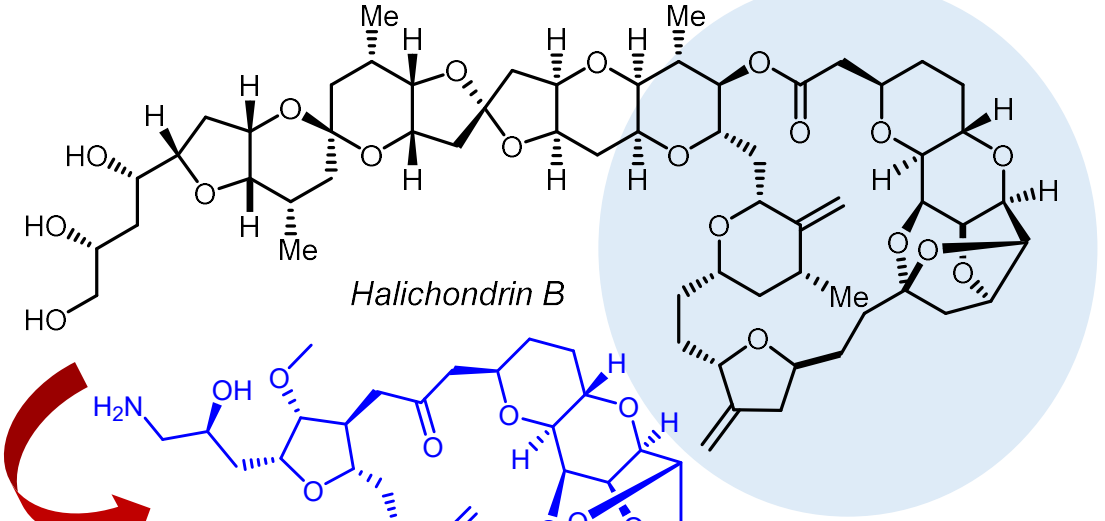
Why natural products!



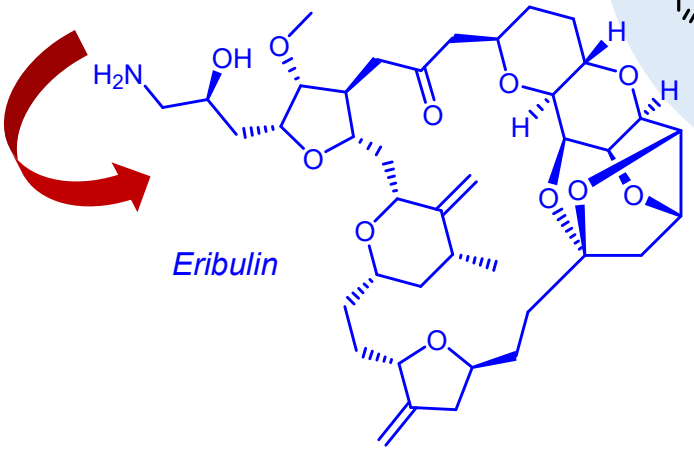
FK506



Salinomycin (for cancer stem cells)



Halichondrin B



Eribulin

- ✓ present complex 3D architectures
- ✓ dense display of stereo-defined groups
- ✓ *challenging task in placing them on the drug discovery path!*
- ✓ excellent track record as small molecule modulators of protein-protein interactions
- ✓ *can serve as a good source of inspiration for developing novel scaffolds*

Building A Chemical Toolbox for "Undruggable" Targets

Our working model

Bioactive Natural Products

Natural Product Inspired Sub-structures

Natural Product Fragments as Sub-structures

Exploring Macrocyclic Chemical Space!

- Cyclic compounds
- Large surface area
- Pre-organization
- Less freely rotating bonds
- Enhanced cell permeation

Key features in our design:

- 3D architectures
- sufficient complexity
- stereochemical and skeletal diversity
- synthesis in a reasonable time-scale
- easy to follow-up medicinal chemistry studies

From Arya Research Team:

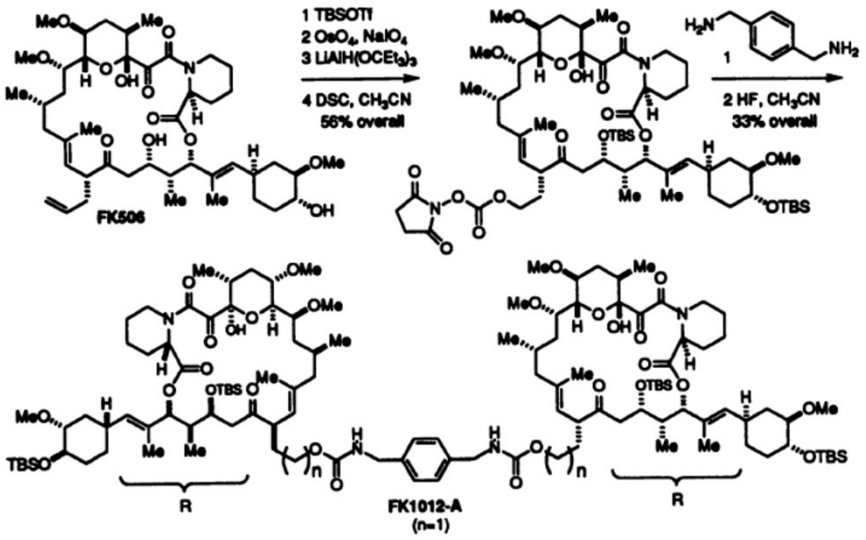
*Chem. & Biol.*163 (2005)
Curr. Opin. Chem. Biol. 247 (2005)
Chem. Rev. 1999 (2009)

Macrocyclic Natural Products and Derivatives

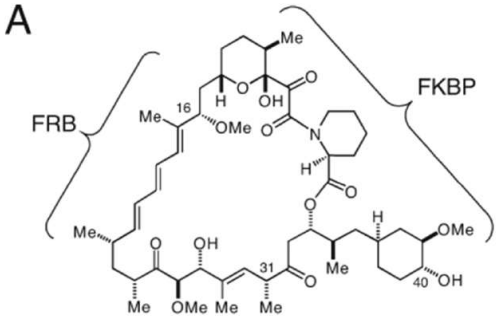
Controlling Signal Transduction with Synthetic Ligands

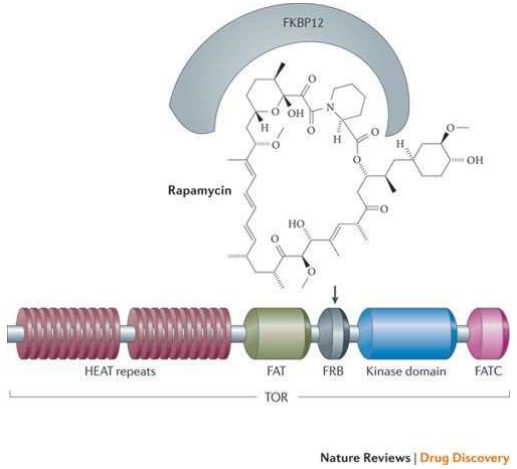
David M. Spencer, Thomas J. Wandless, Stuart L. Schreiber,* Gerald R. Crabtree*

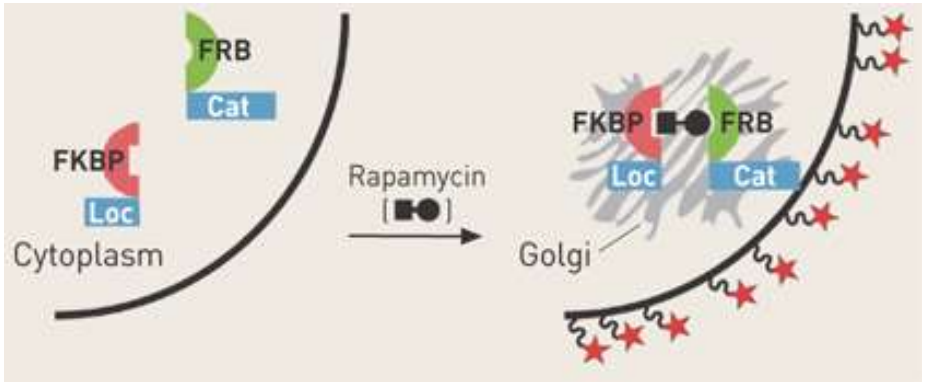
Science, 1993



Classical Example - Rapamycin







Our Early Days!

Diversity-Based Organic Synthesis in the Era of Genomics and Proteomics**

Prabhat Arya,* Doug T. H. Chou, and Myung-Gi Baek

Angew Chem **2001**

RESCUING COMBICHEM

Diversity-oriented synthesis aims to pick up where traditional combinatorial chemistry left off

STU BORMAN, C&EN WASHINGTON

Chem & Eng News **2004**

The natural-product-like compounds produced in DOS have a much better shot at interacting with desired molecular targets and exhibiting interesting biological activity.

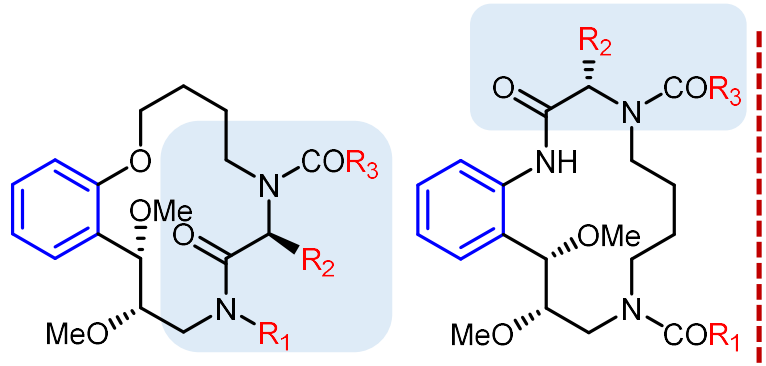
Building Our Chemical Toolbox

Examples of Our Early Work

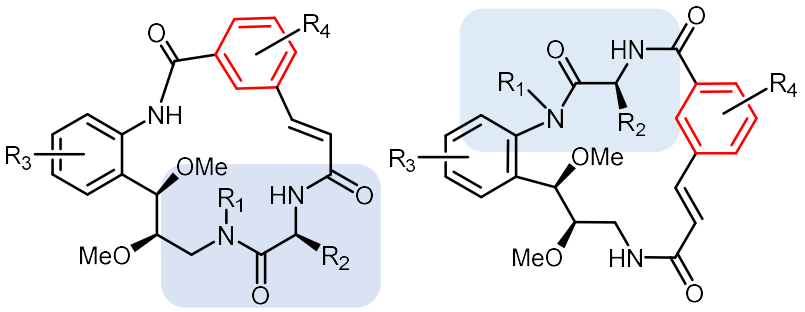
1 Natural Product-Inspired, Functionalized 14- and 17-Membered Rings Macrocyclic Toolbox

2 Indoline / Tetrahydroquinoline (Alkaloids) and Benzofuran (Flavonoids)-Inspired Macrocyclic Toolbox

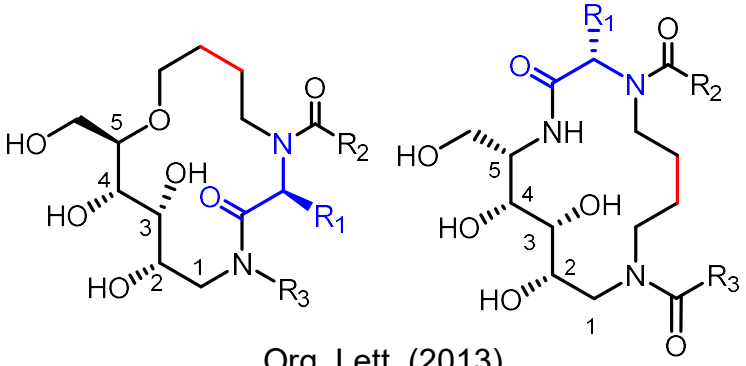
3 Glyco-based Macrocyclic Toolbox



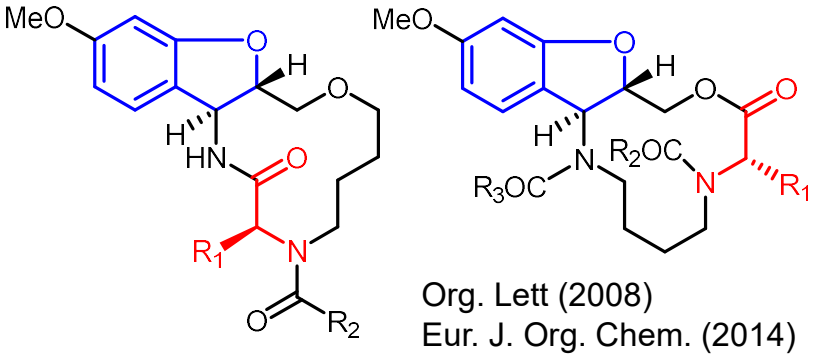
Org. Lett. (2013)



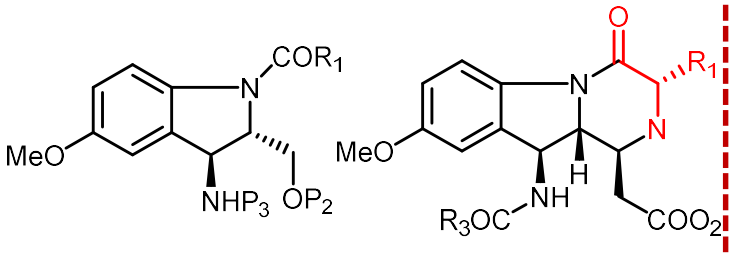
Eur. J. Org. Chem. (2013)



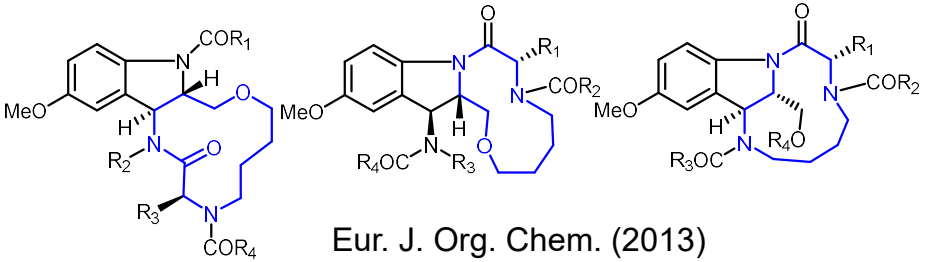
Org. Lett. (2013)



Org. Lett (2008)
Eur. J. Org. Chem. (2014)



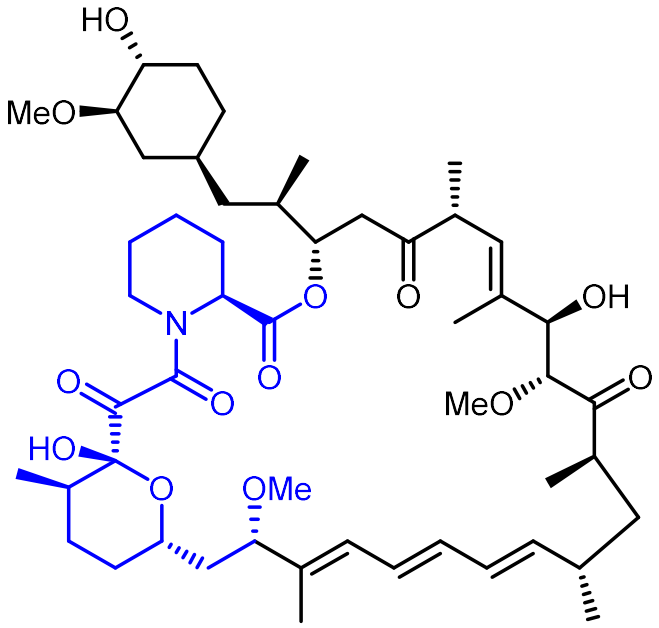
Angew Chem (2005)



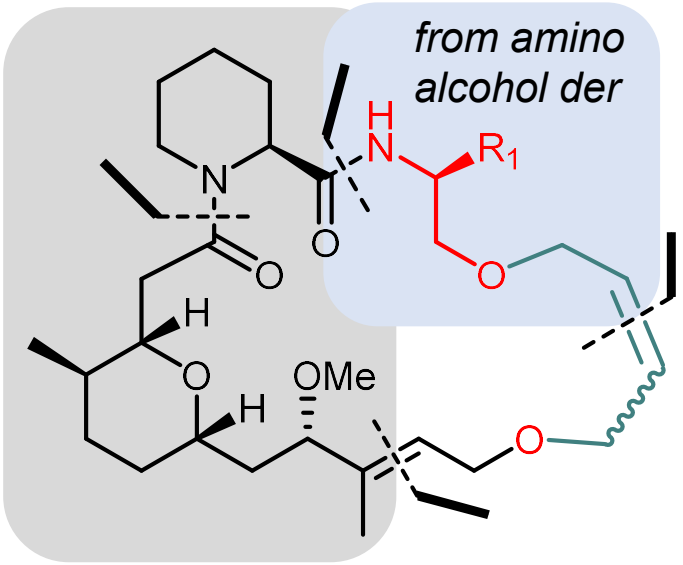
Eur. J. Org. Chem. (2013)

Example 1: Rapamycin fragment-based Macrocyclic Toolbox

Recent Examples

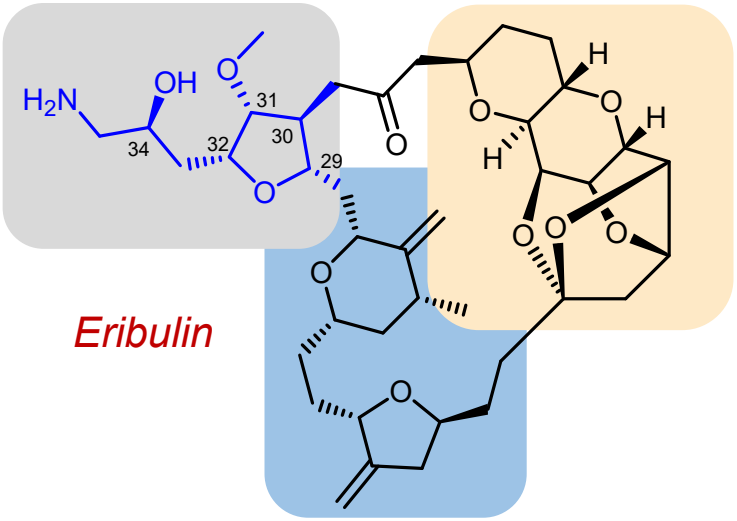


Rapamycin

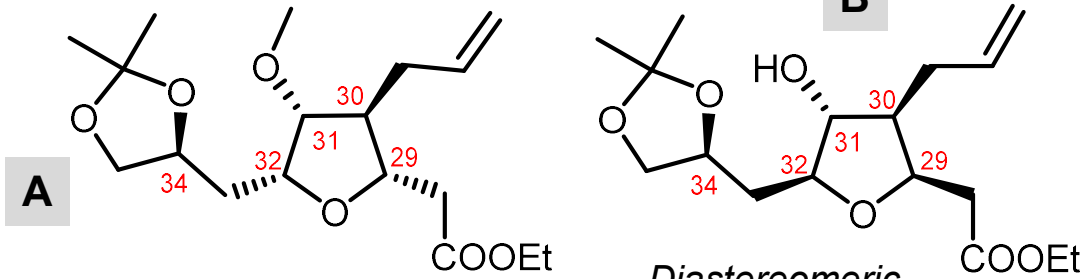


Org. Lett. 480-483 (2015)

Example 2: Macrocyclic Toolbox based on Eribulin Sub-structures

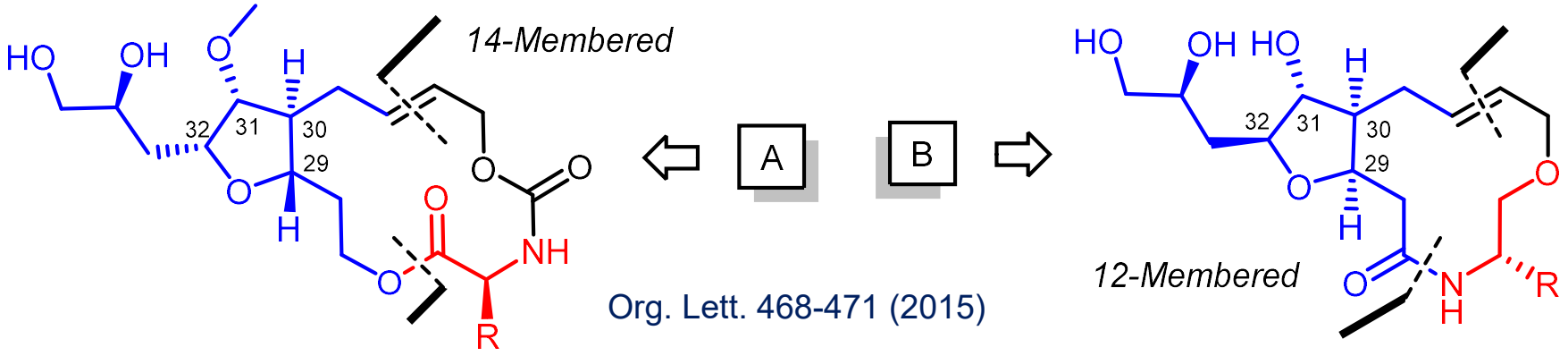


Practical and scalable synthesis



Precise fragment-based

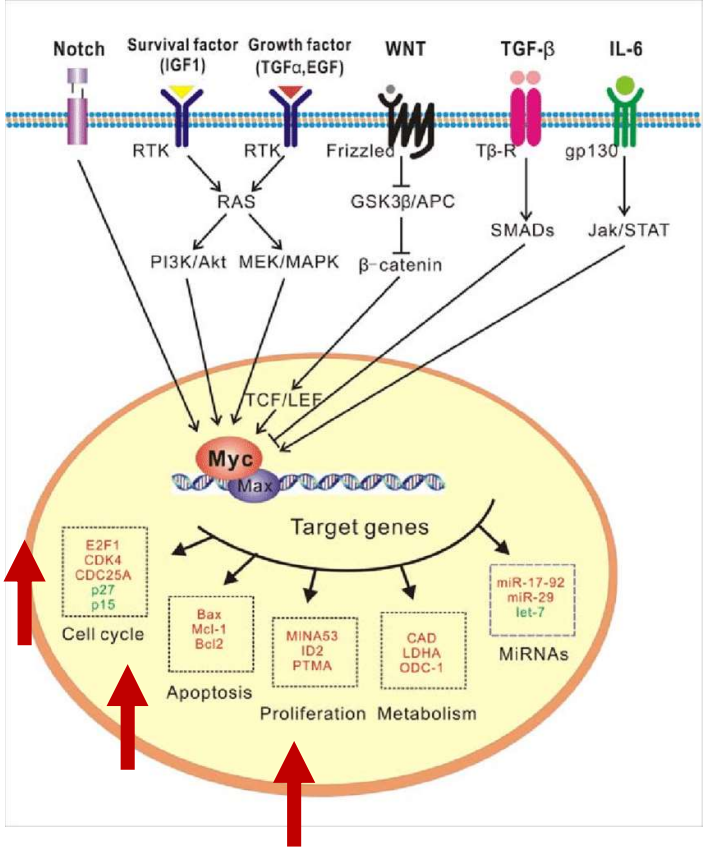
Diastereomeric fragment-based



Org. Lett. 468-471 (2015)

Our Cancer Drug Discovery Journey – A Case Study

De-regulated c-Myc Signaling

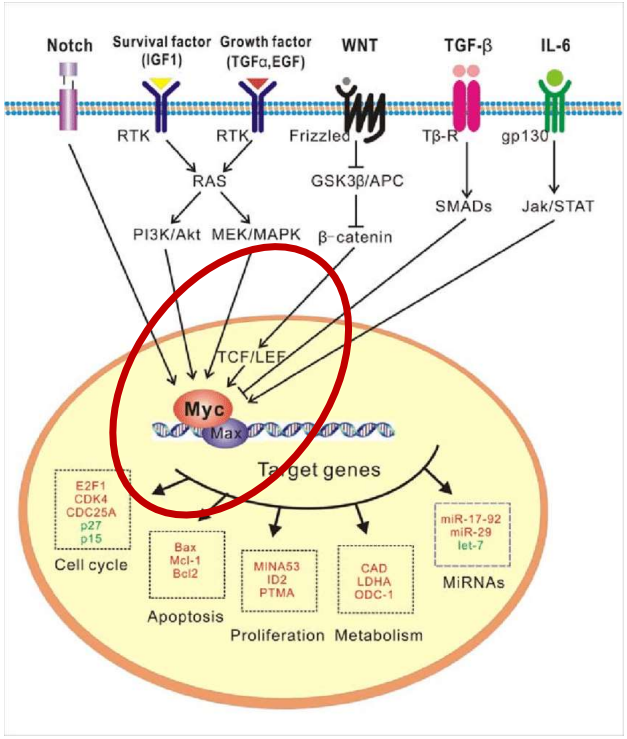


- "*Undruggable*"
- Frequently mutated and overexpressed in many human tumors
- A transcription factor
- Responsible for upregulation of several genes related to cell cycle, apoptosis, and cellular transformation which are directly associated with cancer
- Despite working on this target for more than 3 decades, the current chemistry approaches have not led to producing the effective drug candidates!

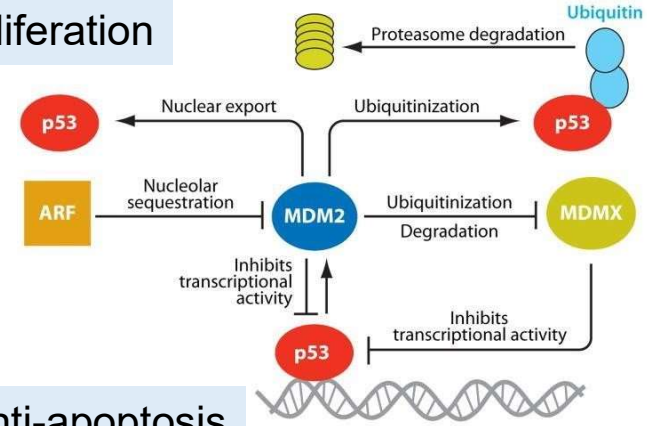
Nat Rev Cancer 2017; Nat Rev Cancer 2008 (Reflecting on 25 Years with Myc)

Myc-related Transcription

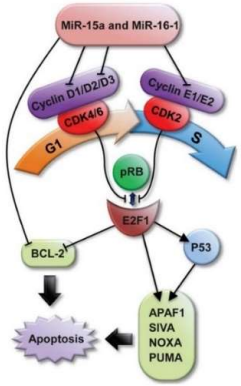
Lead to upregulation of several oncogenes involved in proliferation, cell cycle and anti-apoptosis



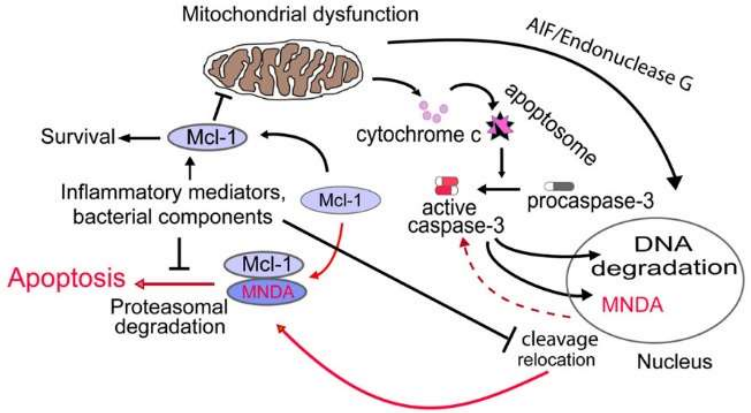
Proliferation



Cell Cycle

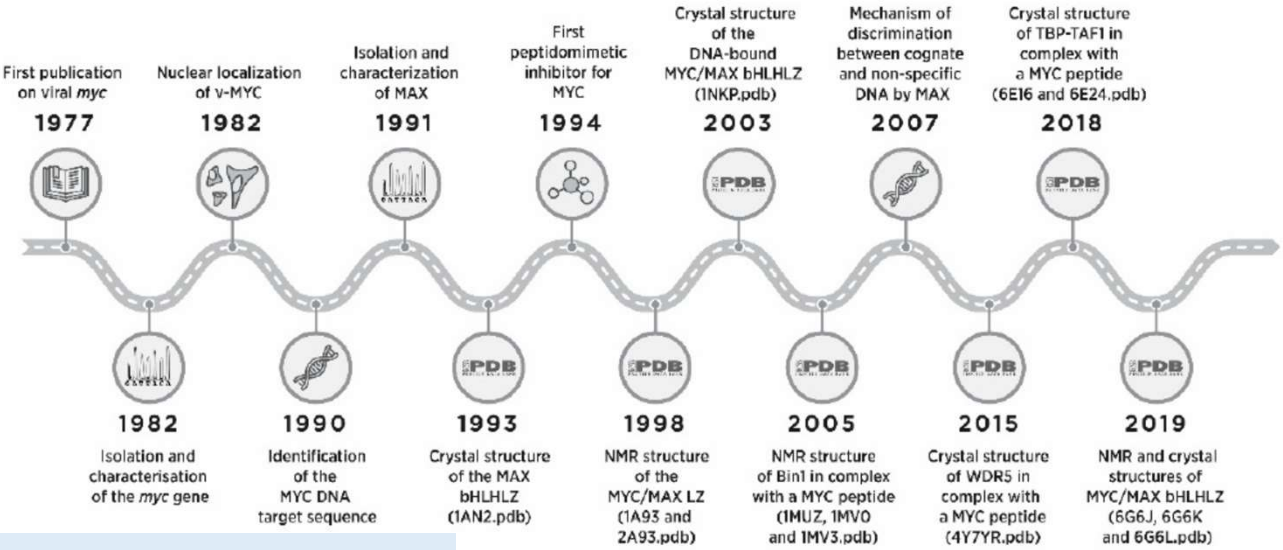


Anti-apoptosis

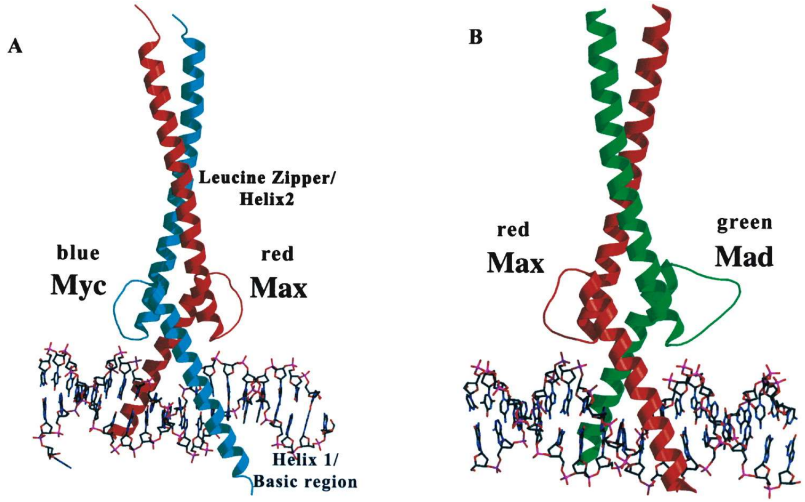


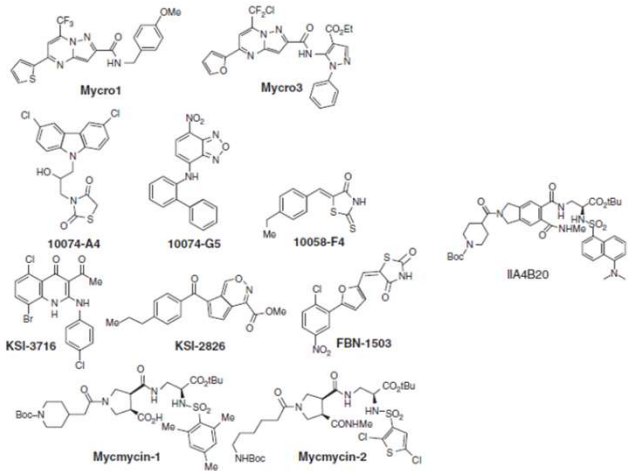
Nat Rev Cancer 2017; Nat Rev Cancer 2008 (Reflecting on 25 Years with Myc)

Myc on Time Scale



Taken from: Cells (2020)





Myc-Max, Protein-Protein Interactions and small molecule inhibitors

Cold Springs Harbor Persp in Med 2018

Why Interested in "*Translation Machinery*"?

SignMod

Classical Approach

Taking care of oncogenic protein targets after they are produced

Vs

Translation Approach

Interfere with the oncogenic proteins (for ex Myc) at their production level!

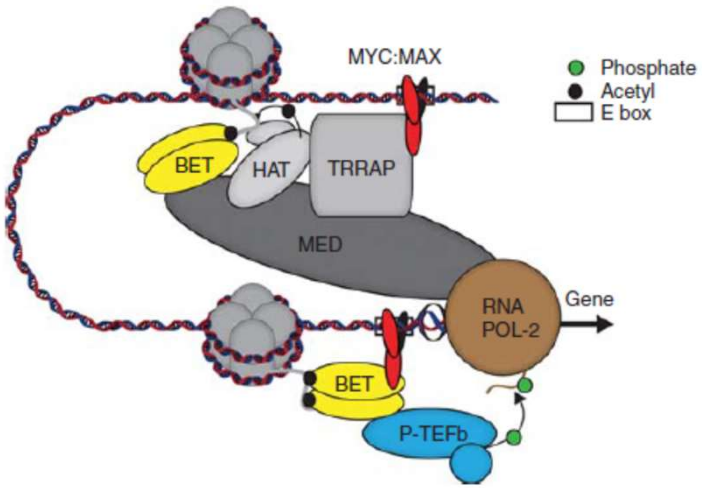
Myc is a transcription factor – play a key role in producing other oncogenic proteins related to proliferation, cell cycle, apoptosis and metabolism

Note: There are no small molecules as direct inhibitors of c-Myc translation!

Some of these oncogenic targets can also be taken care off by inhibiting c-Myc translation

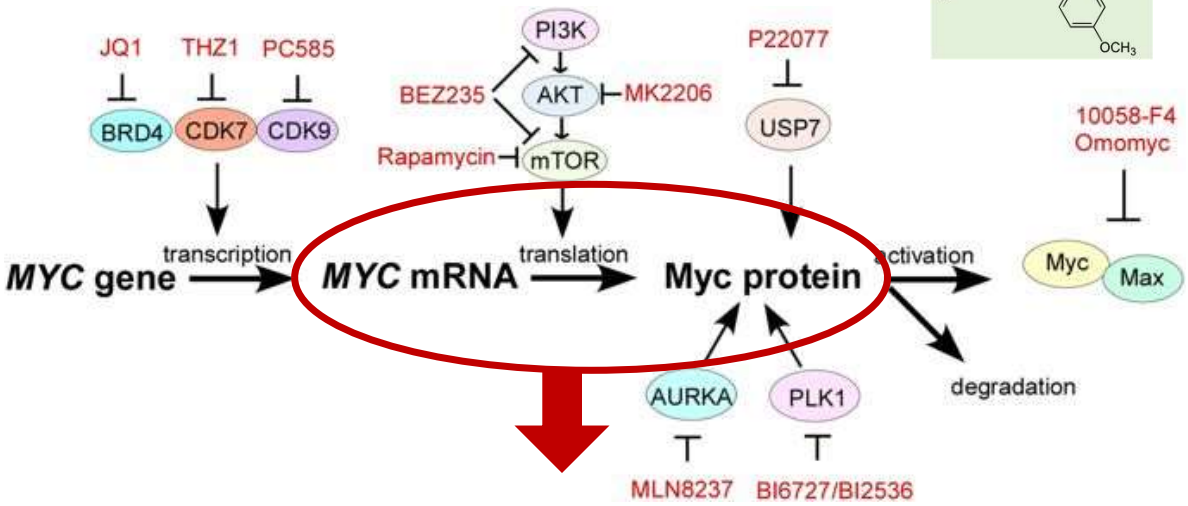
Emerging Approaches to Tackle c-Myc

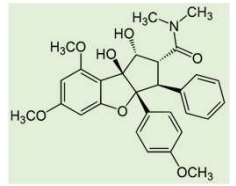
Transcription



Cold Springs Harbor
Persp in Med 2018

c-Myc Translation



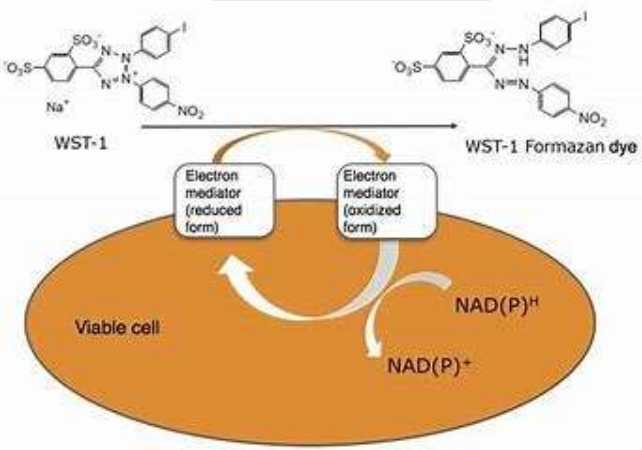


- Only Rocaglamide (natural product) is known as the c-Myc translation inhibitor (in academic literature)
- No small molecules are known in the patent arena!

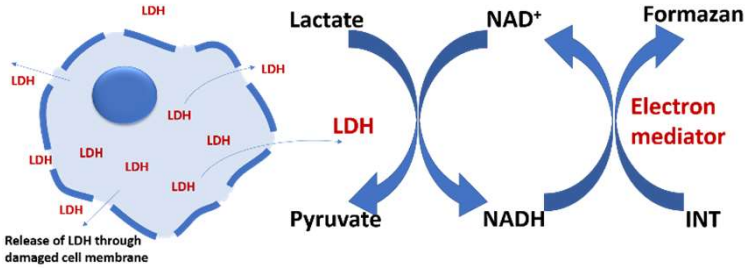
Science Transduction and Targeted Therapy, 2018

Primary Cellular Screening Assays

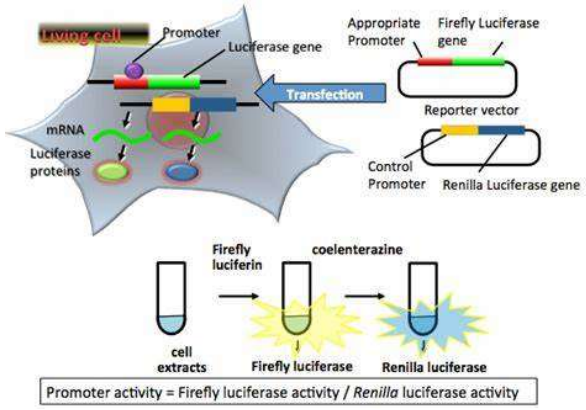
WST1 Assay



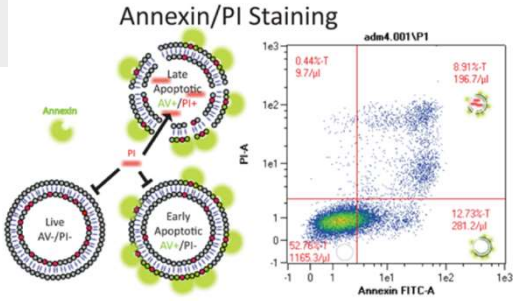
LDH Assay

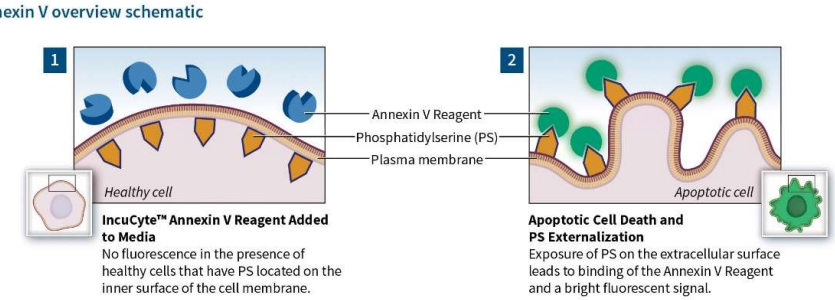


Myc-luciferase Assay



Annexin-V / PI Assay

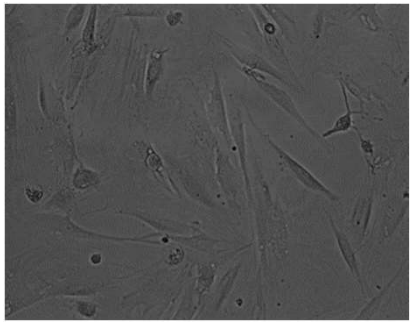




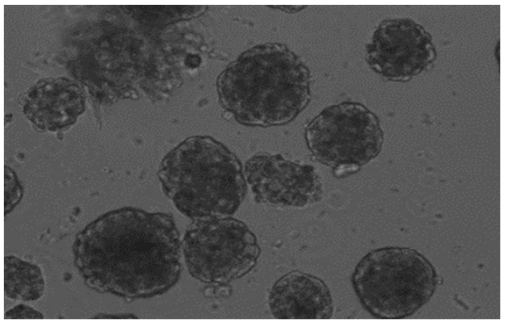
Patient-derived Ex Vivo Models for Secondary Screening

Tumor Biopsy

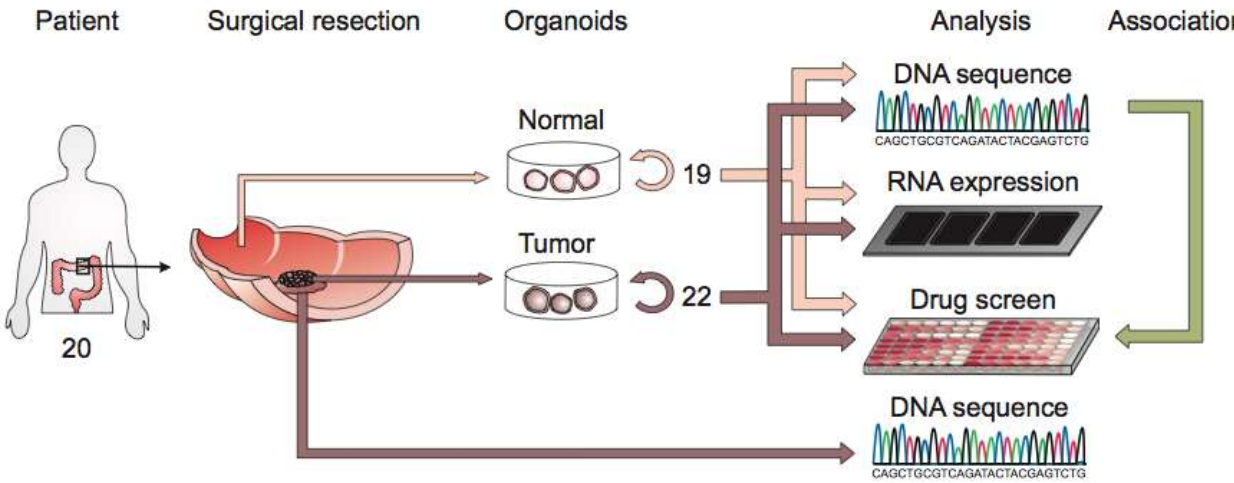
Primary Cancer Cells



Tumorspheres

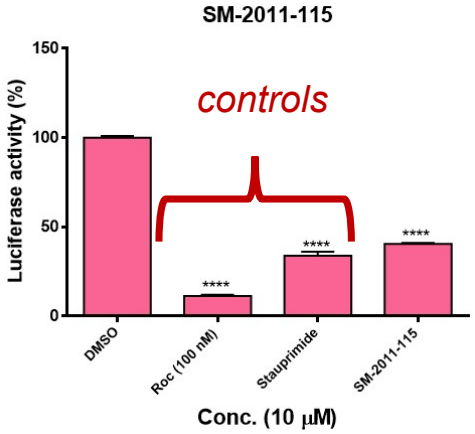


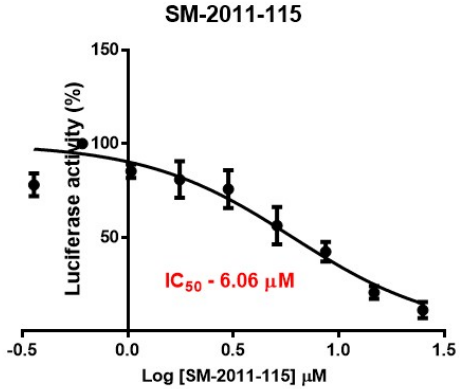
Organoids

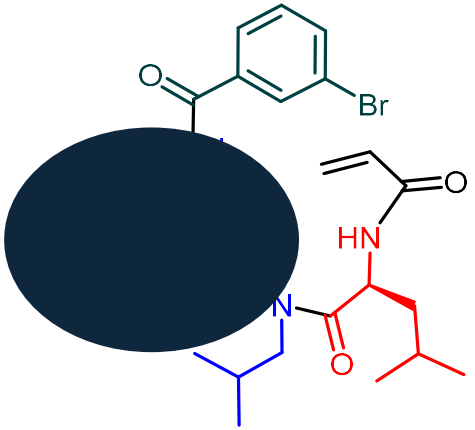


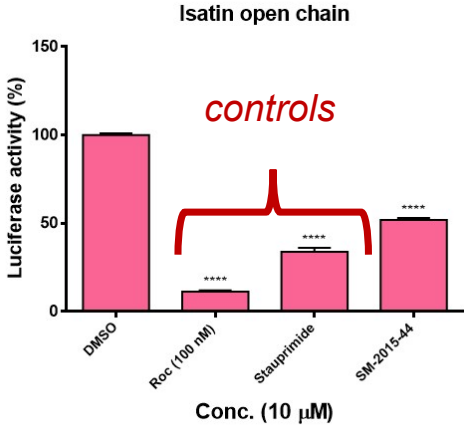
In collaboration with bio-banking, Transcell group, http://transcellonco.science/

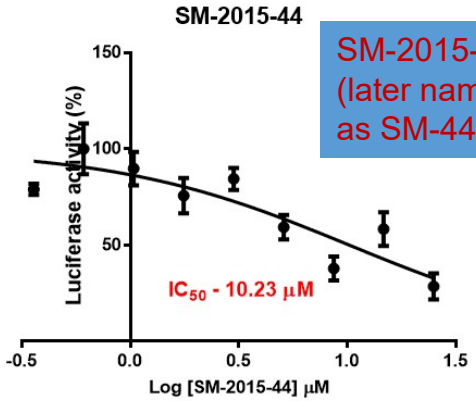
Primary Screen 1: Myc-Luciferase Assay



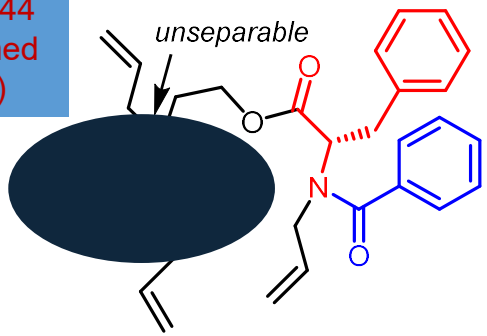






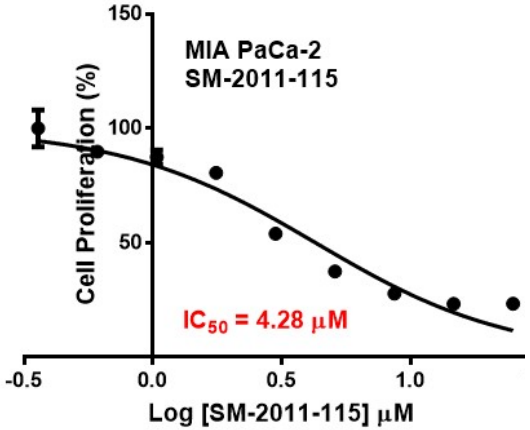


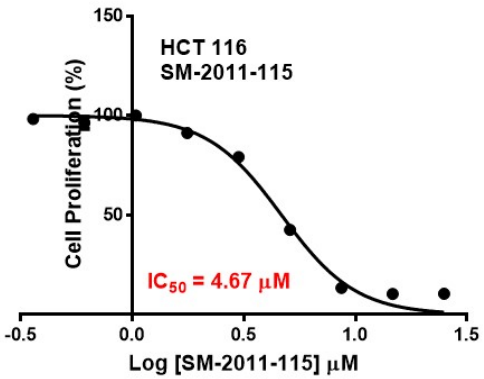
SM-2015-44 (later named as SM-44)

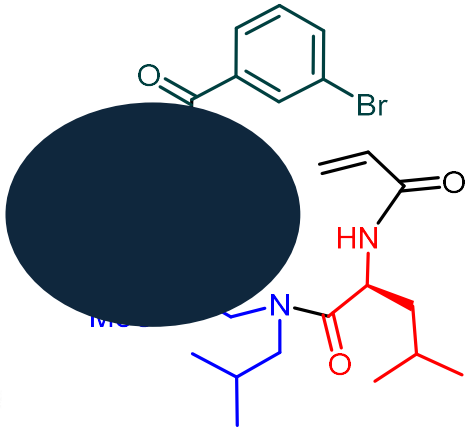


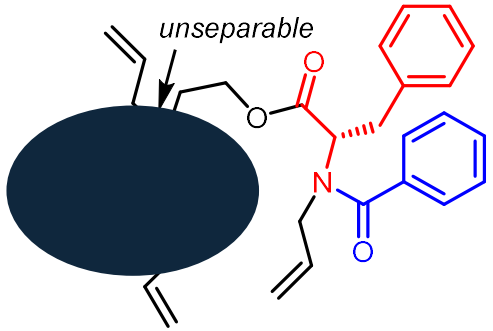
Roc = Rocaglamide

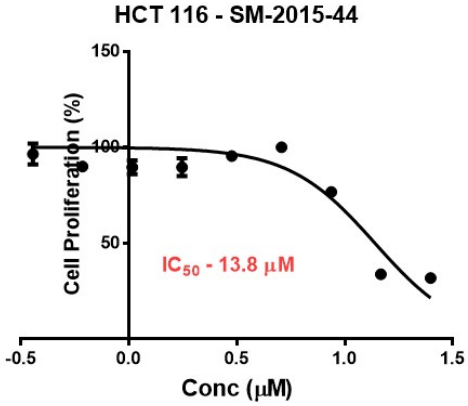
Primary Screen 2: WST1 Assay



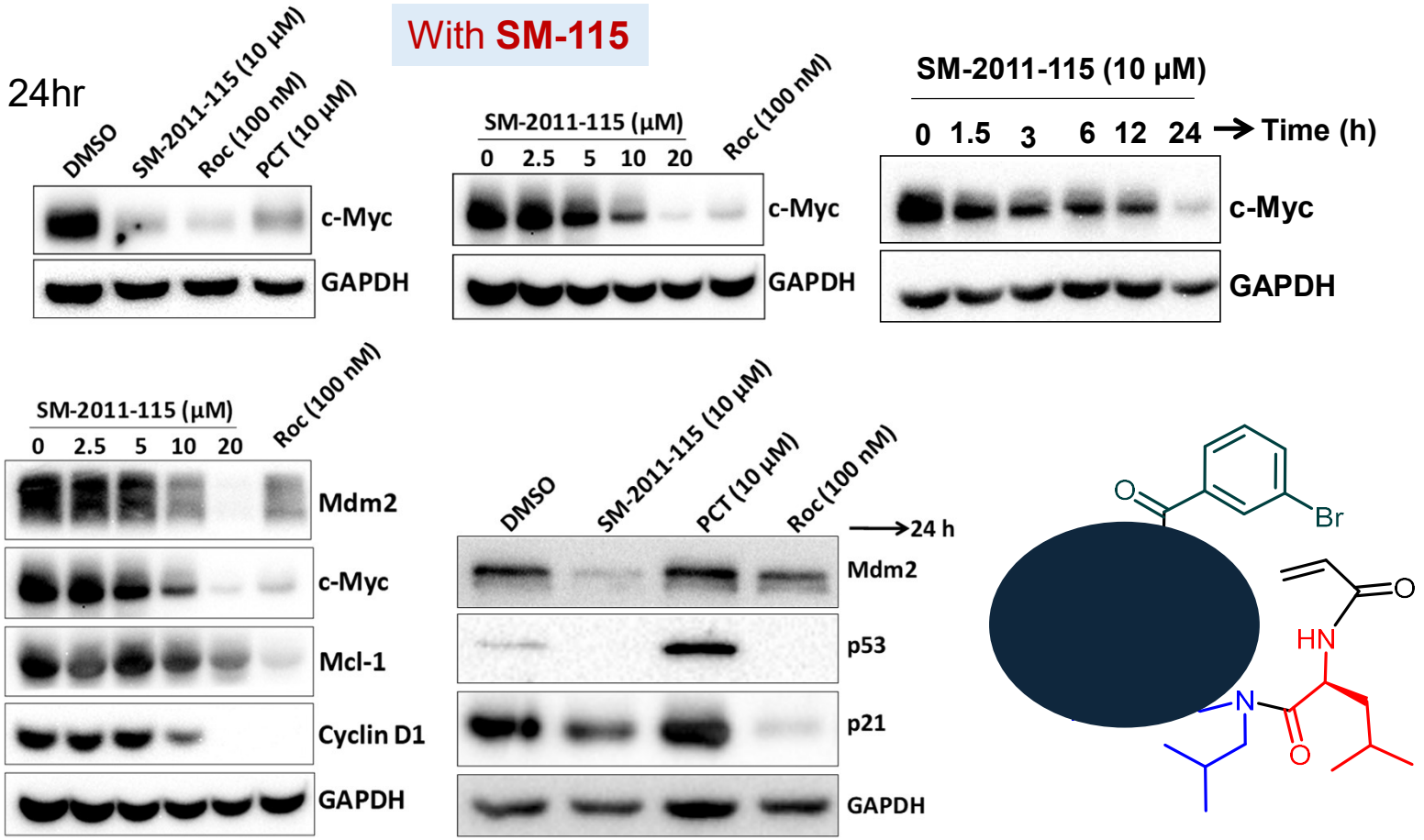






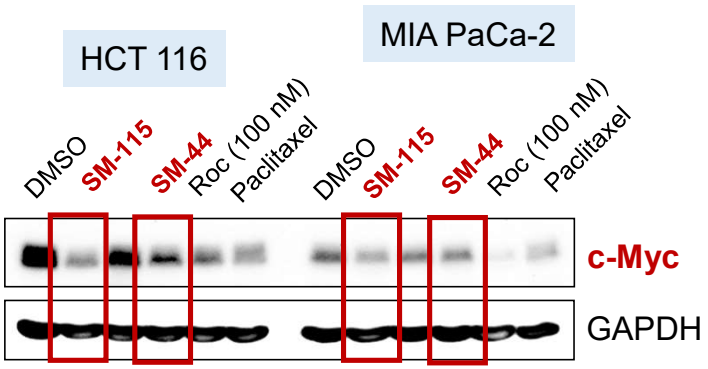


Biochemical Analysis (Western Blots)



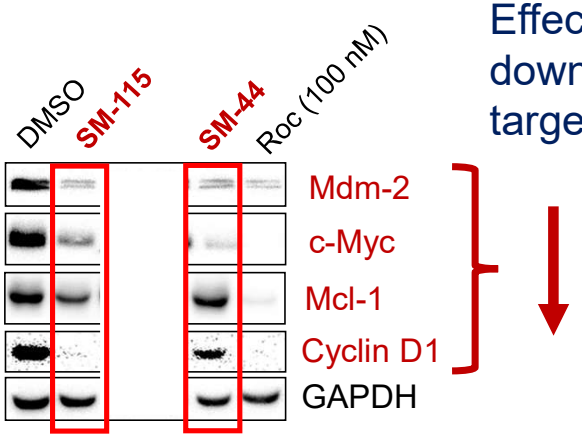
Biochemical Analysis (Western Blots) Contd.

Both molecules hit target



@ 24 hrs, 10uM

Effect on downstream targets of c-Myc



Note: Serious decrease in expression level of c-Myc, MDM-2, Mcl-1 and Cyclin D1!

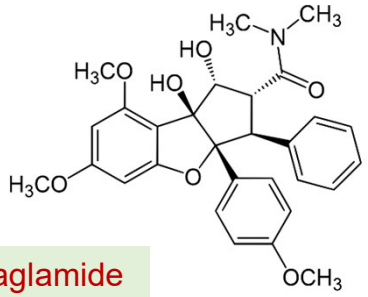
SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

2017

CANCER

Inhibiting the oncogenic translation program is an effective therapeutic strategy in multiple myeloma

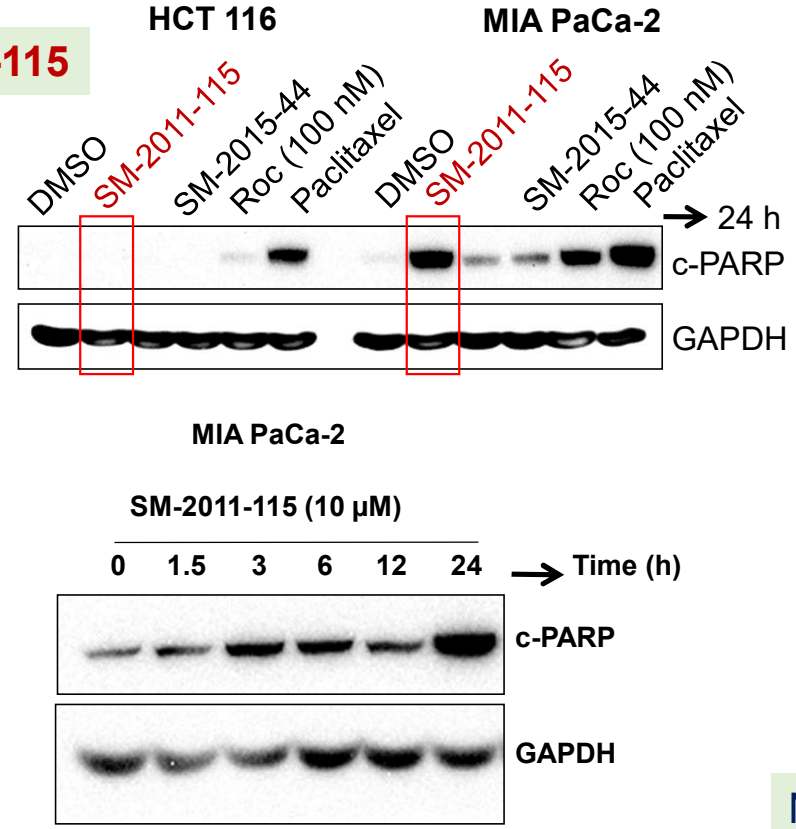
Salomon Manier,[1,2,3]*† Daisy Huynh,[1]* Yu J. Shen,[1] Jia Zhou,[1] Timur Yusufzai,[1] Karma Z. Salem,[1] Richard Y. Ebright,[1] Jiantao Shi,[1] Jihye Park,[1] Siobhan V. Glavey,[1] William G. Devine,[4] Chia-Jen Liu,[1] Xavier Leleu,[5] Bruno Quesnel,[3] Catherine Roche-Lestienne,[3] John K. Snyder,[4] Lauren E. Brown,[4] Nathanael Gray,[1] James Bradner,[1] Luke Whitesell,[6] John A. Porco Jr.,[4] Irene M. Ghobrial[1]†

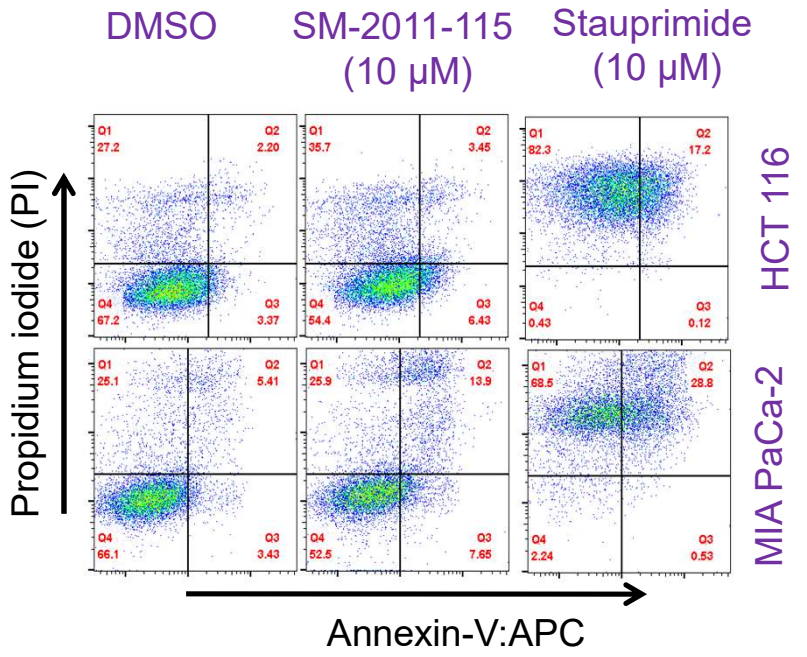


Rocaglamide

Functional Assay: Cell Death

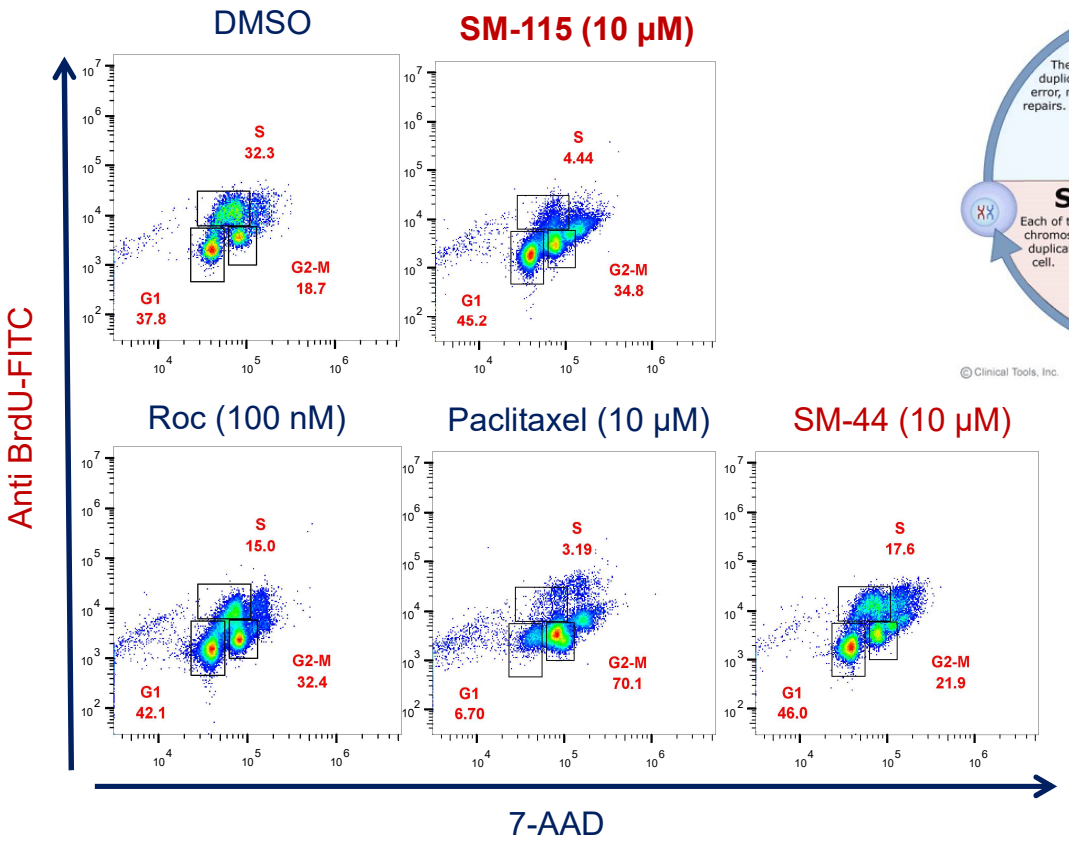
With **SM-115**

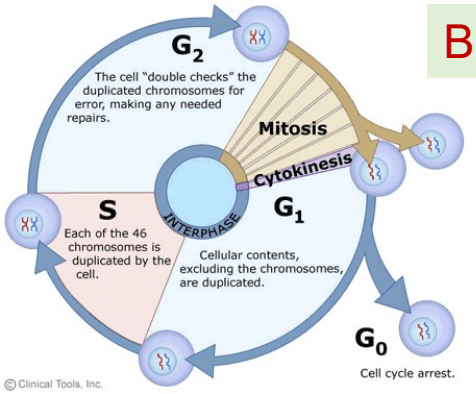




Note: Cell death activation is observed stronger in MIA PaCa-2 cell line than HCT-116

Functional Assay: Flow Cytometry





BrdU Incorporation

Cyclin D1
CDK4

	G1	S	G2/M
DMSO	36.6	32.3	18.6
SM-115	43	**4.44**	34.5
SM-44	43.2	17.6	21.7
Rocaglamide	36	15	31.1
Paclitaxel	6.19	3.19	69.4

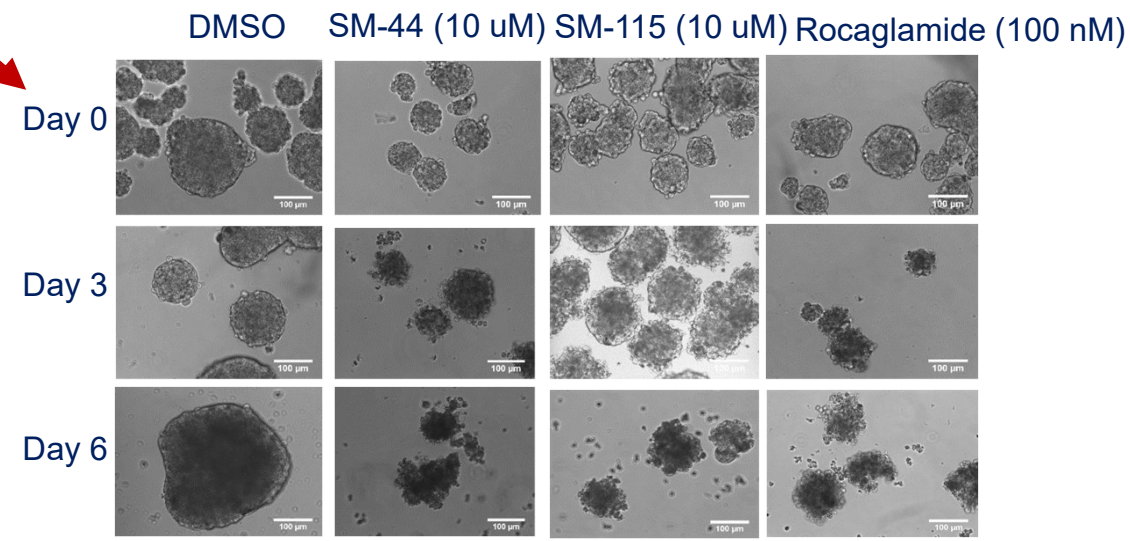
Roc = Rocaglamide

Note: G1 arrest – possible downregulation of Cyclin D1 and CDK4

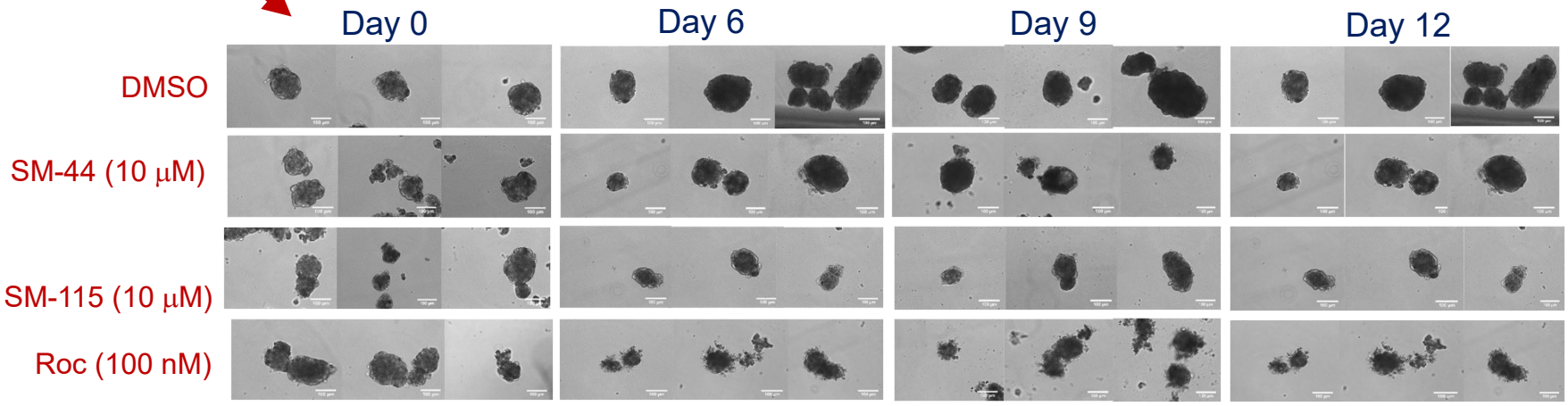
Tumor Efficacy: Effect on Tumorspheres

HCT116-derived Tumorspheres (2nd Gen)

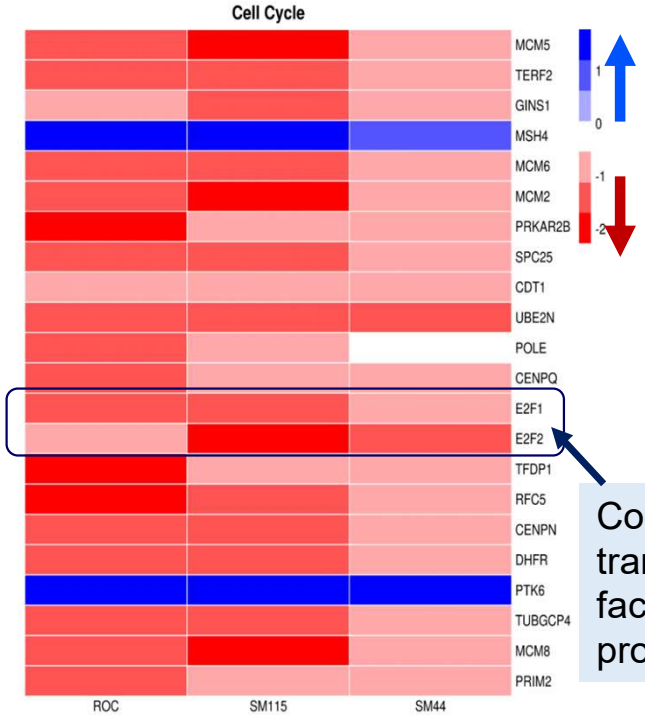
Images from three different tumorsphere sites



Buccal Mucosa Patient-derived Tumorspheres (2nd Gen)



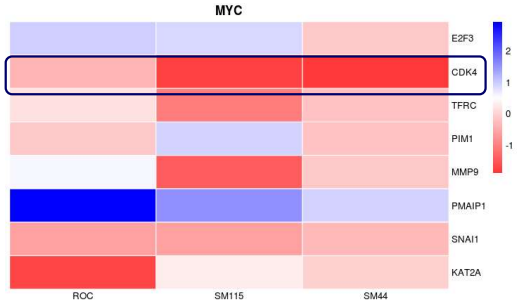
Transcriptomic Analysis



Codes for transcription factor proteins

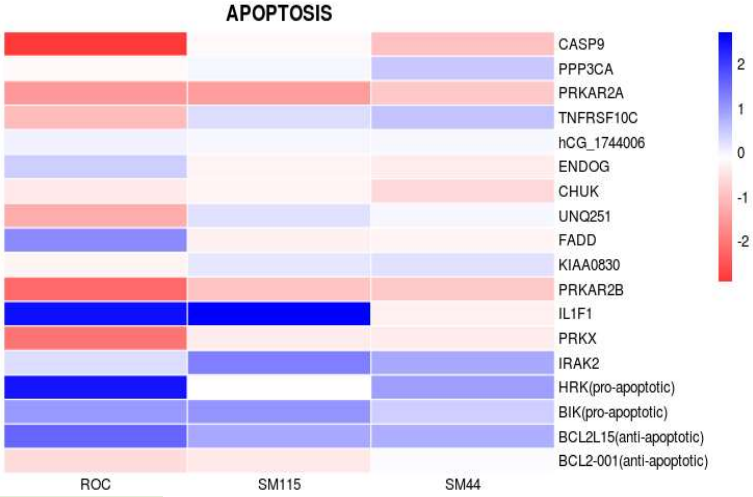
Note: A similar pattern in cell cycle gene set (see ROC vs SM115 and SM44)

Comparison of data with Rocaglamide (ROC) and our two actives (SM115 and SM44)



CDK4 gene crucial in cell cycle from G1 to S phase

Note: Downregulation of CDK4 with SM115 and SM44

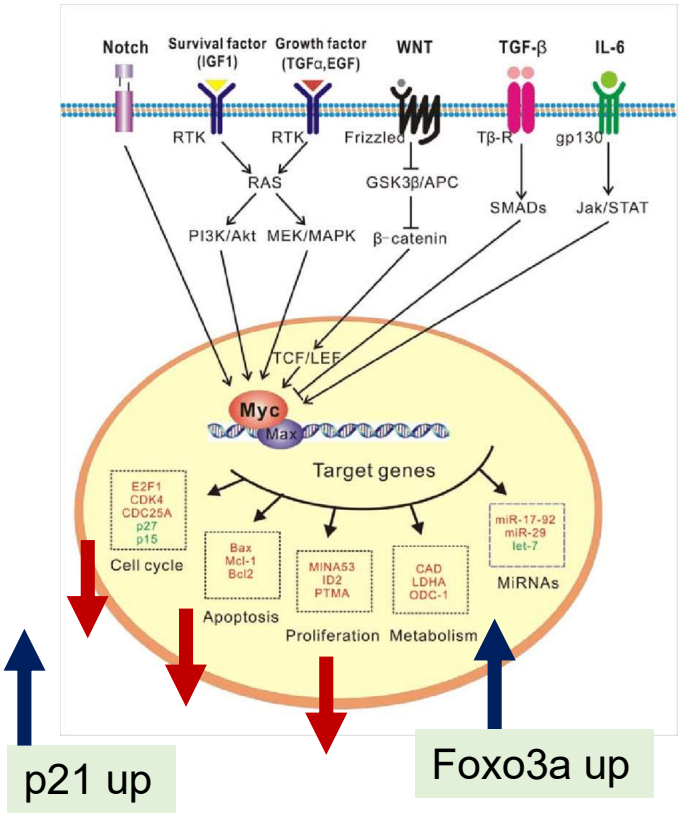


Down regulation of anti-apoptotic gene

Upregulation of pro-apoptotic genes

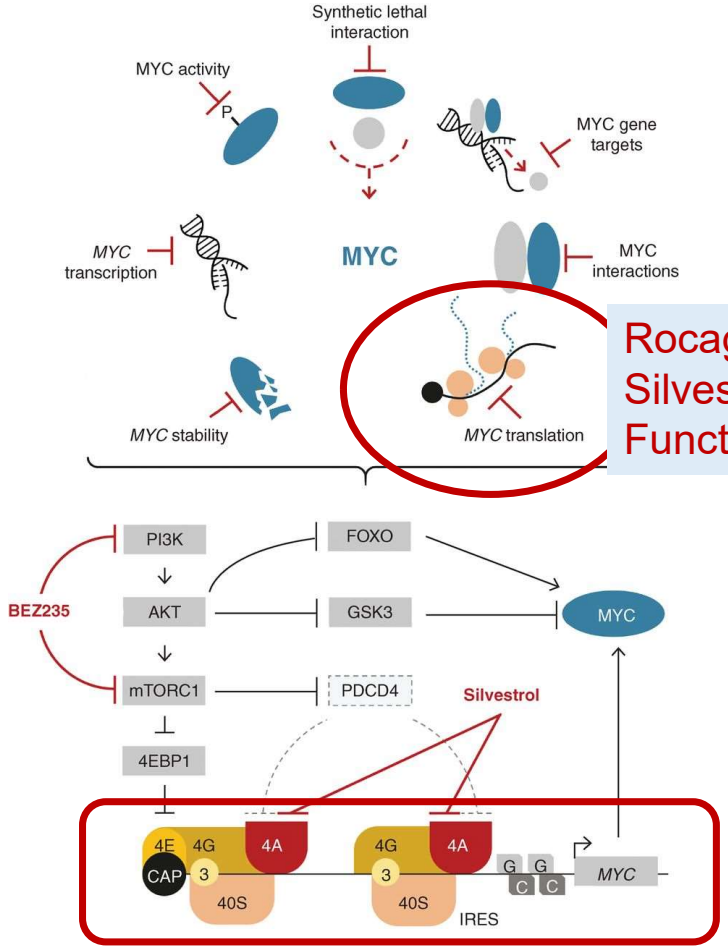
Note: similar upregulation for BIK (pro-apoptotic gene), BCL2-001 (anti-apoptotic gene)

Highlights of Our Program (To-date)



1. Discovered two novel small molecules as c-Myc translation inhibitors.
2. Our actives are effective in inhibiting the target (c-Myc) and its downstream signaling cascade (MDM-2, Mcl-1 and Cyclin D1).
3. Target is associated with apoptosis and cell cycle arrest at the G1 phase.
4. Our two new classes of c-Myc inhibitors are considered as the *functional mimics of Rocaglamide*.
5. Data show tumor efficacy in cell lines and patient tumor-derived tumorspheres models.
6. Data from both biochemical and transcriptional studies indicating possible MOA
7. To our knowledge, there are no small molecules known to date; our work opens up a new direction in the field of c-Myc translation-based cancer drug discovery!

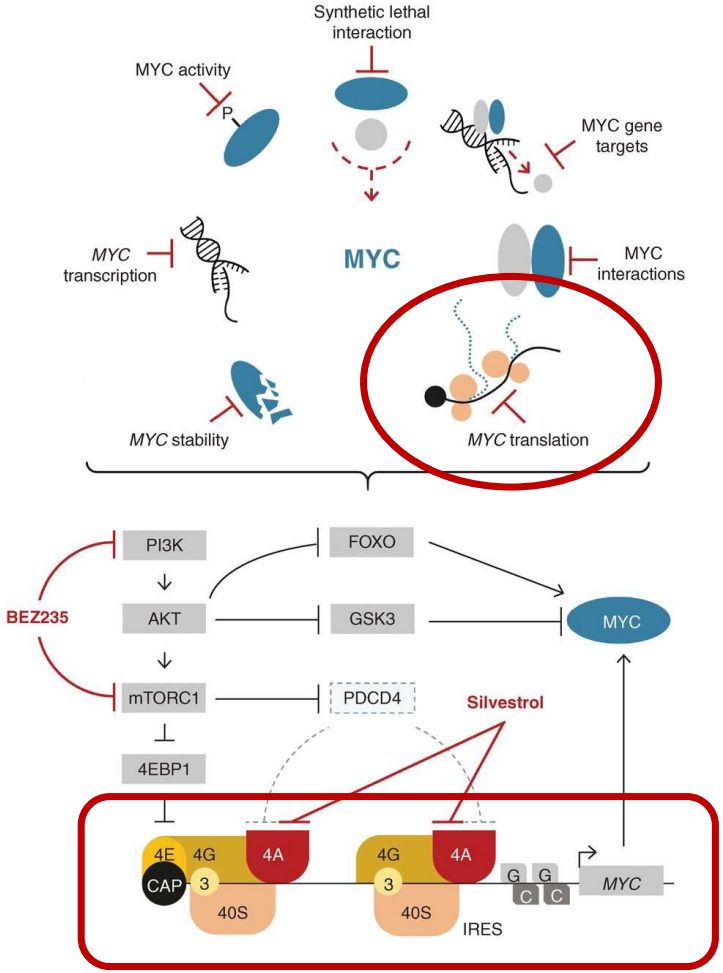
The Next Steps!

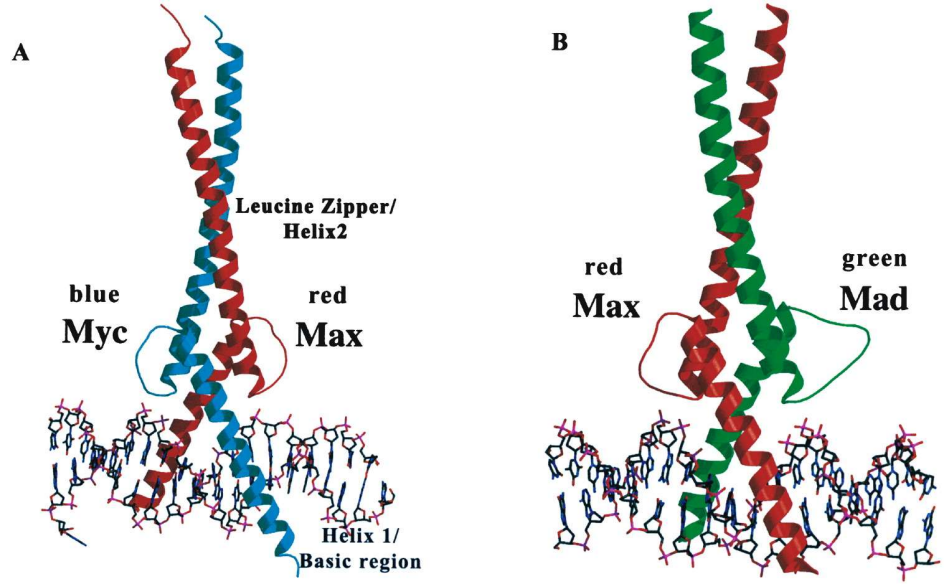


Rocaglamide / Silvestrol-like Functions!

- Optimized lead candidates
- Tox studies / safety profiles
- *In-Vivo* studies
- Precise mode of action?

Which Approach is Clinically More Effective?





The direct inhibitors of c-Myc or small molecule working via Translation / Transcription machinery!

Thank You!

Prabhat Arya
Founder and President

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Former Distinguished Research Professor, Chemistry and Chemical Biology
Dr. Reddy's Inst of Life Sciences, University of Hyderabad Campus