Detection and analysis of potential therapeutic targets for Multiple Myeloma lines

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Contents

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1 Introduction

1 - Introduction

Systems biology and Genomic-scale metabolic modeling

- Metabolism is a **network** of reactions, composed of metabolites and subject to genetic control.
- Mathematical representation of the **biochemical transformations** that occur in cells (GEM-models).
- These models provide a platform to make quantitative and qualitative **simulations and predictions**.
- Models can be produced by constraint-based modeling (**CBM**) and simulations carried out by flux balance analysis (**FBA**).

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1 - Introduction

Road to personalized medicine

- It is possible to model cells from unicellular and **multicellular** organisms, such as humans.
- Different **biological contexts** can be represented via GEMs, useful for studying metabolic diseases.
- Different molecular abnormalities can cause a **similar phenotypic change** (disease challenge).
- What might be a **good strategy** for treating diseases that involve different types of diseased cells?

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1 - Introduction

Goals and scope

- The **overarching objective** of this research is to identify and analyze the most promising therapeutic targets in multiple myeloma (MM) cell lines.
- **Specific goals**:
  - Calculate and check minimal cut sets (MCS) of reactions on a large number of models.
  - Identify MCSs with high MM cancer reach and low toxicities.
  - Analyse the MCSs further to clarify the results obtained.

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2 Materials and Methods

2 - Materials and Methods

Contraint-Based Modeling and Flux Base Analysis

- CBM **represent models** by exploiting physico-chemical and biological constraints.
- CBM incorporates **equations** that assume steady state internal metabolite concentrations and distinguishes between reversible and irreversible reactions.
- FBA detects model states based on optimising **objective functions**, typically biomass production.
- Given a set of modes *T*, a **cut set** (**CS**) is a set of reactions whose simultaneous blocking renders all modes in *T* unfeasible.

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2 - Materials and Methods

Workflow overview

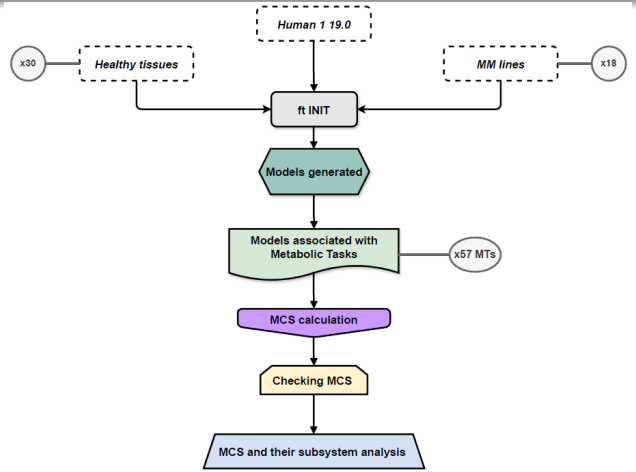

Figura: **Workflow**

2 - Materials and Methods

Constructing models

- *Human-GEM* is considered the most complete model by the community.
- This generic cell model can be used to construct specific models of cells present in healthy tissues or malignant cells (ftINIT algorithm).
- The specific models can be modified to create even more specific models associated with each metabolic task.
- ***Human-GEM*** (1) $--> $ **Specific** models (30 + 18) $--> $ **Metabolic task** models (57 additional)

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2 - Materials and Methods

Computing, checking and analyzing MCS

- **MCS**: Minimal set of reactions whose inactivation renders a metabolic task impossible.
- Here we only have computed MCS consisting in a single reaction (**essential reactions**).
- The minimal cut sets (MCSs) were **reviewed** and recalculated if any errors were detected.
- To **analyse** the MCS we used the *Metabolic Atlas* portal and access to *REST web services*.
- A MCS in a specific model must be **interpreted** as:
  - Toxicities if the model comes from a healthy tissue
  - Targets if the model comes from a multiple myelome line

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3 Results

3 - Results

MCSs obtained from each model

- Firstly, we studied reactions whose blockage involves the death of one of the different tissues.
- In total, there are 441 generic toxicities (MCS from the generic model).
- A total of 912 reactions were identified as toxicities for some healthy tissue and 972 reactions were identified as targets for some MM line.
- Avoiding generic MCS, we obtained:
  - 471 tissue-specific MCS
  - 531 MM line-specific MCS

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3 - Results

Toxicities and toxicity levels

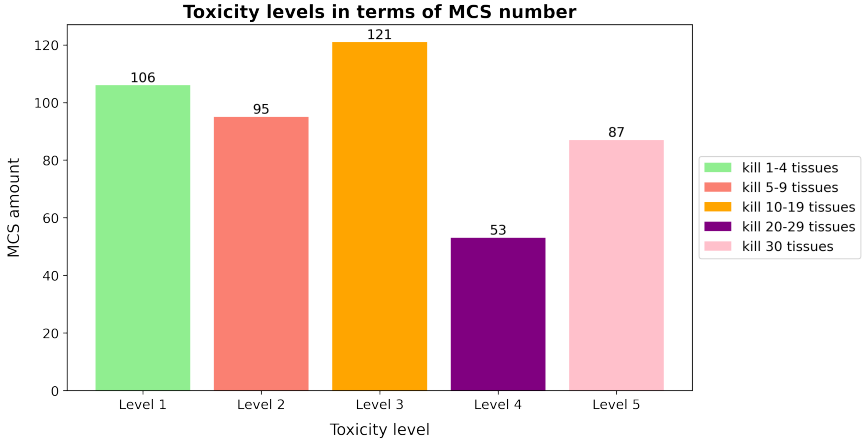

Figura: **Healthy tissues toxicities classification.**

3 - Results

Therapeutic targets encountered for each line

MM lines ID	Total MCS	without generic MCS
KMM1	702	261
AMO1	784	343
HUNS1	663	222
EJM	620	179
RPMI8226	713	272
MM1S	680	239
KMS11	643	202
KMS18	670	229
JJN3	709	268
KMS26	676	235
KMS27	647	206
KMS34	670	229
INA6	710	269
OCIMY7	677	236
KMS20	619	178
SKMM2	683	242
LP1	636	195
L363	711	270

Cuadro: **MCS from each MM line with and without generic toxicities**

3 - Results

Common targets for several MM lines

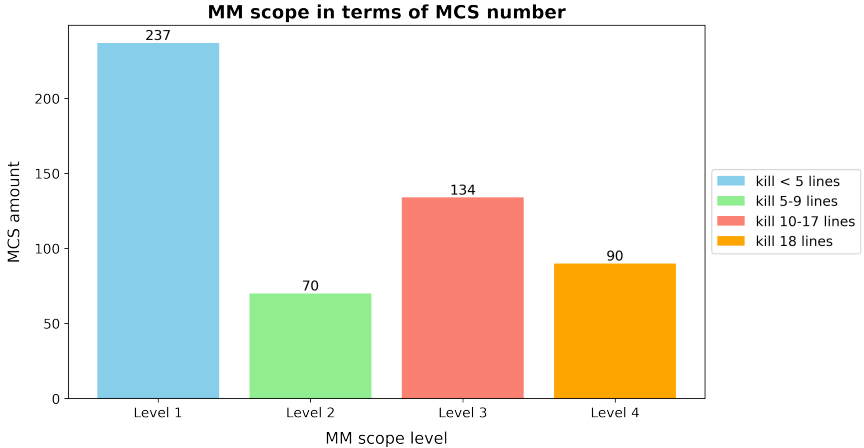

Figura: **MM lines MCS classification.**

3 - Results

MCS for each MM line cosidering toxicities

MM lines ID	Total MCS	without generic MCS	Tox $<$ Level 1	Tox $<$ Level 2
KMM1	702	261	15	28
AMO1	784	343	26	40
HUNS1	663	222	32	47
EJM	620	179	5	23
RPMI8226	713	272	26	43
MM1S	680	239	8	27
KMS11	643	202	19	32
KMS18	670	229	9	23
JJN3	709	268	16	43
KMS26	676	235	8	22
KMS27	647	206	15	33
KMS34	670	229	6	19
INA6	710	269	36	55
OCIMY7	677	236	10	24
KMS20	619	178	13	27
SKMM2	683	242	26	76
LP1	636	195	13	29
L363	711	270	33	51

Cuadro: **MCS from each MM line with low and medium toxicity**

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3 - Results

Common low-toxicity targets in several MM lines

ID MCS	MM lines	Metabolite associated names	Subsystem
MAR06660	17	9-cis-retinoate <=> retinoate	Retinol metabolism
MAR06644	15	9-cis-retinal + H+ + NADH <=> 9-cis-retinol + NAD+	Retinol metabolism
MAR08702	15	9-cis-retinal <=> retinal	Retinol metabolism
MAR04623	16	glucono-1,5-lactone-6-phosphate + H2O − > 6-phospho-D-gluconate + H+	Pentose phosphate pathway
MAR04473	14	6-phospho-D-gluconate + NADP+ − > CO2 + NADPH + ribulose-5-phosphate	Pentose phosphate pathway
MAR02598	12	CoA + O-propanoylcarnitine <=> L-carnitine + propanoyl-CoA	Carnitine shuttle (M)
MAR06386	12	glucose-6-phosphate + Pi − > glucose-6-phosphate + Pi	Transport reactions

Cuadro: **MCSs summary for several lines with low toxicity**

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3 - Results

Combining more than one low-toxicity target

Reaction Pair	Subsystem	MM lines number	Toxicities
MAR06644 MAR04623	Retinol metabolism, Pentose phosphate pathway	18	Blood
MAR06660 MAR04473	Retinol metabolism, Pentose phosphate pathway	18	Blood
MAR06660 MAR04623	Retinol metabolism, Pentose phosphate pathway	18	Blood
MAR08702 MAR04623	Retinol metabolism, Pentose phosphate pathway	18	Blood

Cuadro: **Association of pairs of reactions that kill all lines**

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4 Discussion

4 - Discussion

Discussion approach

- Our analysis will focus on:
  - Investigating further the reactions (MCS) identified as **potential targets**.
  - Identifying which **tasks** are affected by blocking these reactions.
  - Examining in detail the **subsystems** to which these reactions belong.
  - Initiating a search for **pharmacological** treatment.

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4 - Discussion

Metabolic tasks and subsystems

- The impact on tasks varies depending on the line type and the subsystem to which the MCS belongs.

ID MCS	Toxicities	Task affected
MAR06660	Blood	Growth
MAR06644	Blood	Growth
MAR08702	Blood	Growth
MAR04473	Nothing	Growth or others
MAR04623	Nothing	Growth or others
MAR02598	Nothing	Growth
MAR06386	Nothing	Growth or others

Cuadro: **MCSs tasks and toxicities summary**

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4 - Discussion

Common low-toxicity targets in several MM lines

- All those promising MCSs affected belong to 4 metabolic subsystems.

ID MCS	MM lines	Metabolite associated names	Subsystem
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MAR02598	12	CoA + O-propanoylcarnitine <=> L-carnitine + propanoyl-CoA	Carnitine shuttle (M)
MAR06386	12	glucose-6-phosphate + Pi − > glucose-6-phosphate + Pi	Transport reactions

Cuadro: **MCSs summary for several lines with low toxicity**

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4 - Discussion

Transport reactions (TR)

The **exchange of Pi** (hydrogen phosphate) between ER and cytosol seems essential.

- Healthy tissue models usually have more than one (not all) reaction which can exchange Pi.
- **MM models has only one**. This can be either MAR01621 (6 cases) or MAR06386 (the other 12).
- The SLC37A4 gene (related to MM) is associated with the presence or absence of MAR01621.
- When MAR06386 is active, it is an MCS for these MM lines without toxicity. However, this reaction also requires the replacement of glucose-6-phosphate (G6P) and this is **where PPP is involved**.

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4 - Discussion

Pentose phosphate pathway (PPP)

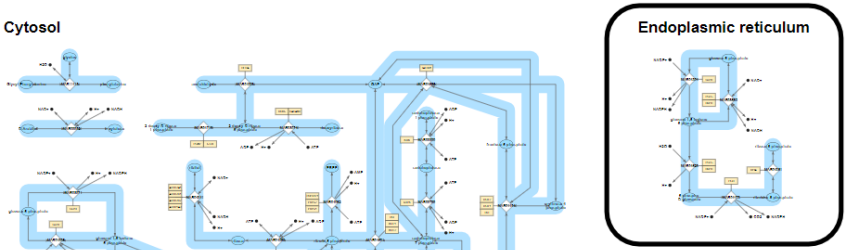

Figura: **Pentose phosphate pathway (*fuente*: Metabolic atlas)**

4 - Discussion

Endoplasmatic reticulum PPP

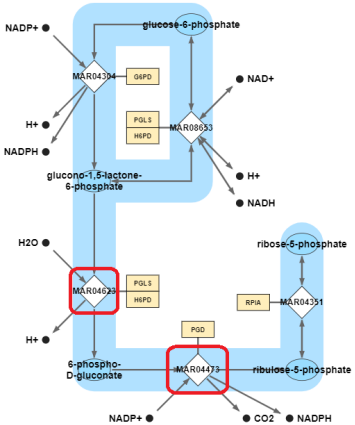

Figura: **Endoplasmic reticulum PPP (*fuente*: Metabolic atlas)**

- There are no major differences between healthy tissues and MM lines models.
- Literature suggests an unknown glucose pathway, triggered by H6PD within the ER of cancer cells.
- Inhibition of G6PD or H6PD expression slows the growth of cancer cell lines.

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4 - Discussion

Retinol metabolism (RM)

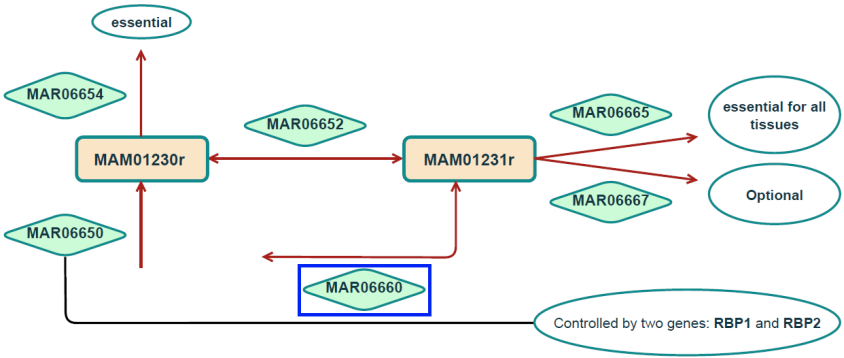

Figura: **Retinol metabolism (specific pathway section)**

4 - Discussion

Carnitine shuttle (Mithocondrial)

- Carnitine is crucial for **facilitating the transport** of long-chain fatty acids into the mitochondria.
- Reduced ALDH6A1-mediated **pro-CoA metabolism** contributes to metabolic remodelling and promotes hepatocarcinogenesis.
- Carnitine **transporters** may form collaborative network for metabolic reprogramming in cancer.

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4 - Discussion

Genes associated with low toxicity targets

ID MCS	Ensembl	Entrez	Name	Symbol
MAR06660	-	-	-	-
MAR06644	ENSG00000157326	10901	dehydrogenase/reductase 4	DHRS4
	ENSG00000135437	5959	retinol dehydrogenase 5	RDH5
	ENSG00000265203	5949	retinol binding protein 3	RBP3
	ENSG00000187630	317749	dehydrogenase/reductase 4 like 2	DHRS4L2
	ENSG00000139547	8608	retinol dehydrogenase 16	RDH16
	ENSG00000170786	195814	short chain dehydrogenase/reductase family 16C5	SDR16C5
	ENSG00000198099	127	alcohol dehydrogenase 4 (class II), pi polypeptide	ADH4
	ENSG00000025423	8630	hydroxysteroid 17-beta dehydrogenase 6	HSD17B6
	ENSG00000140522	6017	retinaldehyde binding protein 1	RLBP1
MAR08702	-	-	-	-
MAR04473	ENSG00000142657	5226	phosphogluconate dehydrogenase	PGD
MAR04623	ENSG00000049239	9563	hexose-6-phosphate dehydrogenase/glucose 1-dehydrogenase	H6PD
MAR02598	ENSG00000157184	1376	carnitine palmitoyltransferase 2	CPT2
	ENSG00000095321	1384	carnitine O-acetyltransferase	CRAT
MAR06386	ENSG00000281500	2542	solute carrier family 37 member 4	SLC37A4

Cuadro: **Interesting MCS genes associated**

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4 - Discussion

Potential drugs associated with low toxicity targets

Target name	Gen symbol	Target chEMBL ID	Molecule drug name
Carnitine palmitoyltransferase 2	CPT2	CHEMBL3238	PIOGLITAZONE ROSIGLITAZONE
Prostaglandin-H2 D-isomerase	PGD	CHEMBL4334	INDOMETHACIN KETOROLAC IBUPROFEN NAPROXEN
Glucose-6-phosphate translocase	SLC37A4	CHEMBL3217398	CHLOROGENIC ACID

Cuadro: **chEMBL information coincidences with diseases**

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4 - Discussion

Drug cheking against the experimental literature

- The thiazolidinediones group is effective in diabetes mellitus 2 but not in cancer.
- Non-steroidal anti-inflammatory drugs (NSAIDs) against cancer has been the subject of research.
- Indomethacin has been described as having anticancer properties.
- **Chlorogenic acid** (**CGA**) has been associated with cáncer but it has to be also considered as toxicity.

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5 Conclusion

5 - Conclusion

End point

- This work has shown the importance of **considering toxicity** while looking for targets in MM.
- The cancer lines analyzed appear to have very **specific subsystems of attack**.
- The endoplasmic reticulum seems to be an **essential organelle** for cancer cells.
- **H6PD** and **SLC37A4**, which control some of these promising reactions, are highly relevant in cancer.
- The most **promising treatment** is chlorogenic acid, which acts against SLC37A4.

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5 - Conclusion

Future prospects

- The **models are limited** in terms of biological information due to their experimental nature.
- In the short term searching minimal cut reaction sets of **length 2** or more, involving **genetic MCS** and combine it with the reactions (**mixed MCS**), or to include many more different lines.
- In the long term, try to model cells of the **immune system**, modelling **connections** between cells and include the **environment** modelling.

Despite its limitations, using GEMs to identificate metabolic vulnerabilities in cancer cells to find therapeutic targets, promises to be an **efficient technique** in personalized medicine.

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Thank you for your attention

Questions?

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