# Repurposing drugs for glioblastoma multiforme: designing a swift and rational passage from bench to bedside

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**Abstract**

Glioblastoma Multiforme (GBM) is the most frequent and fatal brain tumor and is characterized by very poor prognosis with a limited overall survival. There is thus a compelling need for innovative and effective therapeutic approaches.

Since the development of new drugs is a process presently characterized by an immense increase in costs and development time, drug repositioning is gaining significance in clinical pharmacology, allowing faster and less expensive delivery of potentially useful drugs from the bench to the bedside. Indeed, in GBM, where a number of old drugs is now considered for clinical use, often in association with the first-line therapeutic intervention. Repurposed drugs offer a cost-effective advantage in addition to an already-established pharmacokinetic and pharmacodynamic profile. Now, the refinement of the molecular mechanism(s) of action of some old drugs through up-to-date technologies is paving the way for their rational use in the therapeutic approach of GBM as well as other cancer types.

Using established and primary human GBM cell lines, we are exploring in depth the pharmacological effects of several drugs amenable of repositioning in GBM employing different technological procedures. Recently, the results obtained suggested us to submit three clinical trials with repurposed/repositioned drugs combined with the current optimal GBM therapeutic approach (Stupp protocol, 2005).

The spiraling costs of new antineoplastic drugs and the long time required for them to reach the market demands a profoundly different approach to keep lifesaving therapies affordable for cancer patients. In this context, repurposing can represent a relatively inexpensive, safe and fast approach to GBM treatment.

## We are using two main approaches to identify the MoA of selected drugs amenable of repurposing in GBM therapy

### 1. Reverse-Phase Protein microArrays (RPPA)

**RPPA is a powerful, antibody-based technique that allows simultaneous profiling of diverse key regulatory cellular factors. RPPA was originally developed to investigate cancer cell signaling and is designed for relative and multiplexed quantification of specific cellular proteins along with their post-translational modifications, allowing a fine measurement of the functional state of selected cell signaling regulators**

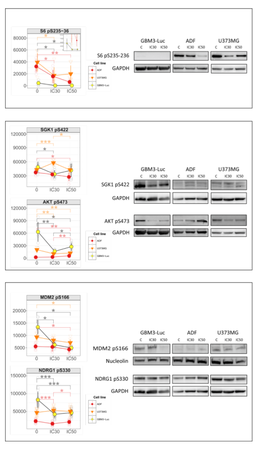
**In the context of repurposing, RPPA is an efficient tool to delineate the effects of drugs on several, cancer-related cellular pathways.**

### The orphan drug SI113

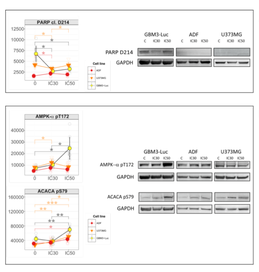
**SI113 is a small molecule identified by virtual screening of a molecular library with respect to the SGK1 crystal structure. SGK1 is a structural and functional analogue of AKT. SI113 is an "orphan" drug, but provided noticeable results in blocking cancer growth in vitro and in vivo.**

**We explored the pharmacological effects of SI113 via RPPA, with the aim to define the overall status of several signal transduction pathways under the effect of this kinase inhibitor**

**mTORC1, mTORC2, AKT and SGK1 activity**

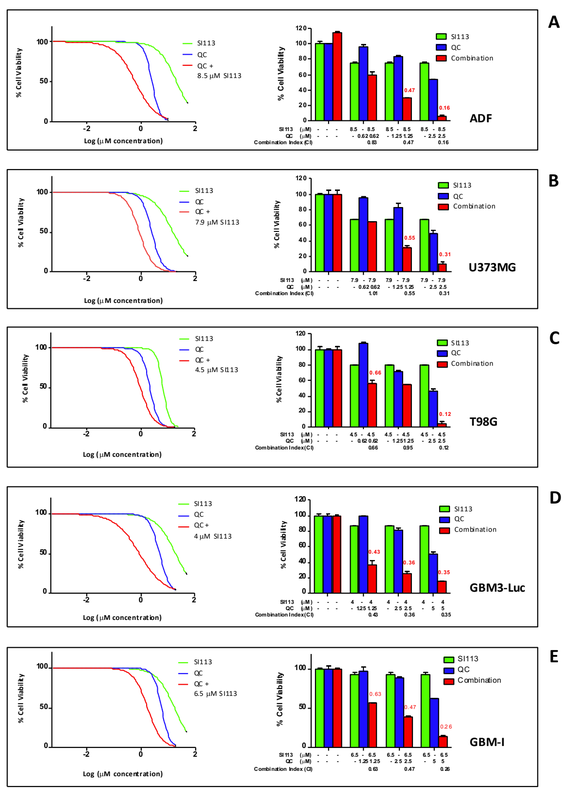


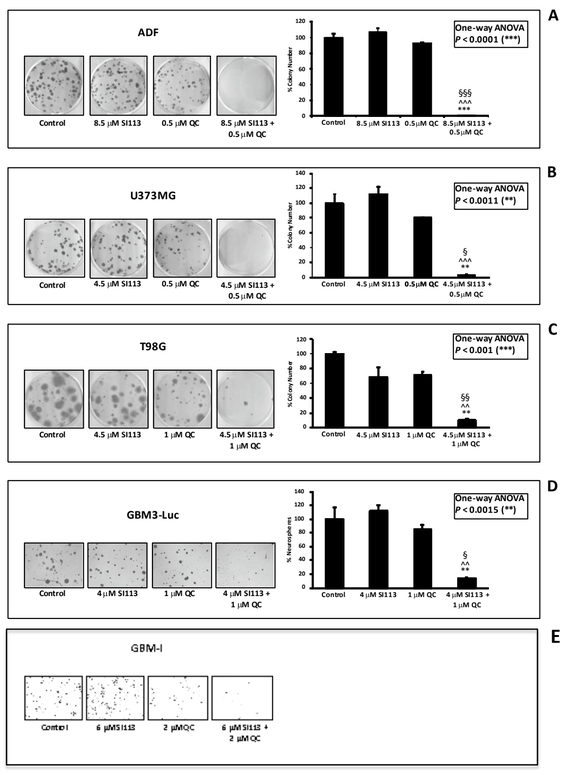

**Apoptosis and autophagy**




**RPPA results.** SI113 inhibits mTORC1 activity in all three GBM cell lines and inhibits mTORC2, AKT, SGK1 activity in neurospheres only, where, in addition, apoptosis was drastically inhibited and autophagy stimulated by the presence of this compound

**SI113 plus autophagy inhibitors**




**Cell Viability.** Established and primary GBM cell lines were exposed to solvent(s) (Control), SI113, QC or their association . The results show that the combined effect of SI113 and QC was synergistic in restraining GBM growth

**Clonogenic Assay.** Established and primary GBM cell lines were exposed to solvent(s) (Control), SI113, QC or their association . The results show that the combined effect of SI113 and QC was highly effective in restraining GBM cell invasiveness

### 2. Activity-Based Protein Profiling (ABPP)

**ABPP is a technology capable of determining the activity of several enzymes in complex biological systems using site-directed covalent probes aimed at assessing the functional state of specific classes of enzymes.**

**ABPP is gaining importance as a uniquely powerful post-genomic method for monitoring protein function in addition to the molecular effects of drugs.**

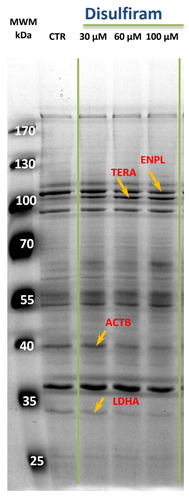
**ABPP proposes a variety of molecular probes able to monitor several enzyme activities, e.g. kinase, protease, serine hydrolase, metalloproteinase, cysteine protease, caspase, deubiquitylase, as well as Cytochrome P450.**

**The use of this technology is often combined with Mass Spectrometry (MS) analysis. In clinical pharmacology, ABPP is particularly useful for understanding drug-target interactions, identifying new therapeutically relevant targets as well as off-target effects and, in addition, providing important information to assess a precise drug concentration required to hit (or not) secondary drug targets.**

**Essentially, RPPA evaluates the biological consequences of the drug activity, while ABPP identifies the physical target(s) of a drug, Indeed, the MoA of a drug cannot be predicted solely based on the detailed spectrum of known drug targets or simply on the effects of a drug.**

### Disulfiram

**Disulfiram is an acetaldehyde dehydrogenase (ALDH1) inhibitor, blocking the conversion of acetaldehyde into acetic acid, thus playing a key role in cancer bioenergetics. Being ALDH1 an activator, through AMPK, of cellular energy metabolism, treatment of sensitive cells with disulfiram results in energy depletion and acetaldehyde accumulation that produces cellular toxicity**




- Cell line: ADF (glioblastoma multiforme)
- Cell lysate incubated for 10 min with disulfiram at the concentrations indicated
- Lysate enriched for ATP-binding proteins and analyzed via 1D SDS-PAGE
- Coomassie staining
- Analysis and Band picking
- MS identification of picked bands

TERA - P55072 (TERA_HUMAN) Transitional endoplasmic reticulum ATPase. Mass: 89.3 kDa.

ENPL - P14625 (ENPL_HUMAN) Endoplasmin. Mass: 92.5 kDa.

ACTB - P60709 (ACTB_HUMAN) β-actin. Mass: 41.7 kDa..

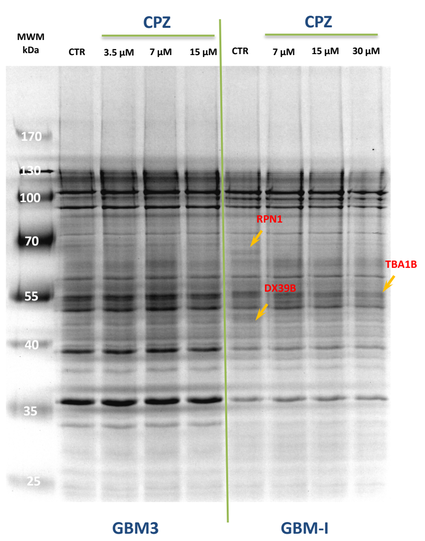
LDHA P00338 (LDHA_HUMAN). Lactate dehydrogenase. Mass: 36.7 kDa.

In this setting, disulfiram increases the activity of TERA and decreases the activity of endoplasmin, β-actin and lactate dehydrogenase, an enzyme involved in bioenergetics and located at the intersection between glycolysis and Krebs cycle

*Presently, we are working at the functional significance of these results*

### Chlorpromazine

**Chlorpromazine (CPZ) belongs to the class of tricyclic antipsychotic agents and acts as an antagonist on dopamine receptors D2 (DRD2). Recently CPZ has been demonstrated to have at least two further MoAs: a) inhibitor of the mitotic kinesin-like protein KIF11; b) inhibitor of the AKT/mTOR axis, thus eliciting autophagic cell death in GBM cancer cells**




RPN1 - P04843 (RPN1_HUMAN) Protein glycosyltransferase subunit 1. Mass: 68.5 kDa.

TBA1B - P68363 (TBA1B_HUMAN) Tubulin alpha-1B chain. Mass: 50.1 kDa.

DX39B - Q13838 (DX39B_HUMAN) Spliceosome RNA helicase DDX39B. Mass: 48.9 kDa.

*Single-experiment results. These data need to be validated*

### Conclusions

**Most of the data here described for these drugs clearly highlight the need for exhaustive basic and translational research to elucidate often poorly understood biological mechanisms underlying the effect of a medication. This is a mandatory step in order to allow the translation to clinical application.**

**Current knowledge demonstrates that often it took decades, or also centuries, to unravel the full complexity of the interplay between a drug and biological systems. At this point, basic and preclinical research can generate proof-of-concept results to stimulate and promote the generation of robust proof-of-concept clinical trials**

***Advantages of drug repurposing:*** **Resources have already been spent to develop these drugs and their resuscitation for similar or novel indications can lead to cost-savings and avoidance of risks related to drug development. Indeed, the knowledge of their well-investigated pharmacokinetic and pharmacodynamic characteristics can spare most of the studies directed to assess drug dosage, safety and side effects.**

**In addition to the above noted advantages of repurposed drugs to diminish expenses and development time to effectively reach the bedside, repurposing should be considered an invaluable opportunity to treat patients when there is no approved therapy or when a patient has exhausted all available treatment options.**

***Obstacles to drug repurposing:*** **A common characteristic shared by most of these medications is that all of them are inexpensive drugs for which patents have not been submitted, have expired or are about to expire, thus making them lacking of monetary incentives for their off-target development. This is paradoxically an obstacle, making it difficult to obtain funding for investigative research.**

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