Metabolic discovery platform:“Seahorse-Bravo”

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INTRODUCTION

The regulation of energy metabolism is very complex and involves a large number of signaling pathways and molecular effectors. Recent development in biology revealed that cancer cells can rewire most metabolic pathways, revealing novel means of bioenergetic regulation (**Figure 1**). For instance, it was shown that C-MYC oncogene controls glutamine-supported cellular respiration and lipid synthesis or that glycolysis bottlenecking by PKM2 promotes de novo serine biosynthesis. With the recently ascertained role of mitochondria in cancer biology, the search for novel regulators of mitochondrial respiration was performed using genetic means, as CRISPR-CAS9 or shRNA libraries, or pharmacological means such as drug-libraries. There is a growing need in academia and industry to dispose technology solutions enabling the discovery of mitochondrial respiration regulators (genes or compounds) using standardized and reproducible high-precision methods. **The aim of this unprecedented CELLOMET-Agilent collaboration is to evaluate the quality and the efficiency of coupling the Agilent Seahorse XF96 Analyzer extracellular flux analyzer with the Agilent Bravo Automated Liquid Handling Platform.**

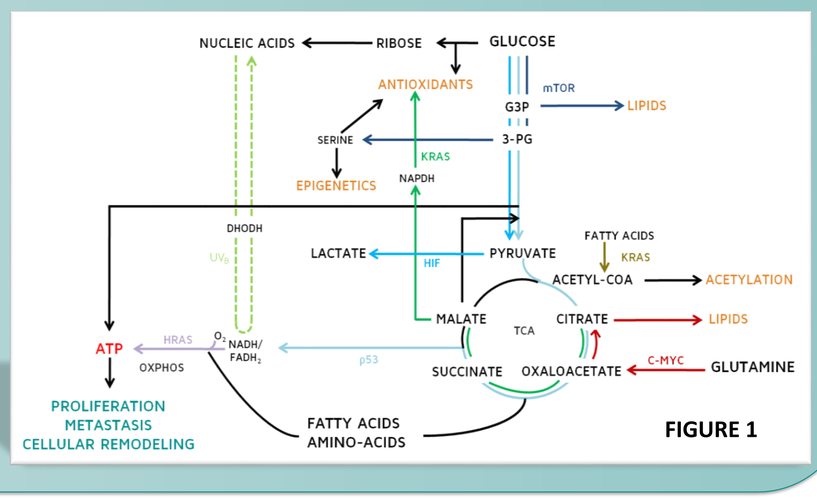


FIGURE 1

Agilent Technologies

Agilent Bravo → Automated pipetting platform

Agilent Seahorse XF96 Analyzer → Metabolism analyzers

Metabolic Science for health

Mitochondrial physiology | Metabolic remodeling | Bioenergetic therapy

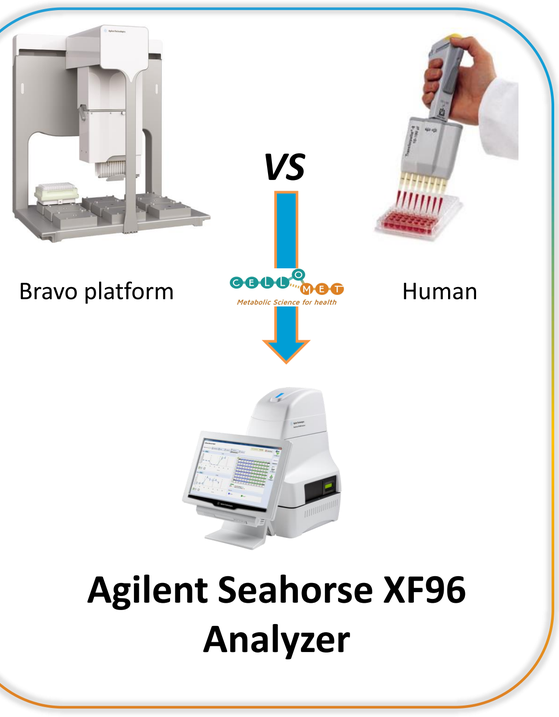
→Experts in the functional and molecular investigation of cellular metabolism

Objective 1 : Decipher the metabolic profile of two cancer cell lines of variable bioenergetics. (→ Seahorse analysis)

Objective 2 : Compare the performance of the Bravo Platform against human pipetting (→Evaluation of the Z prim factor).

Objective 3 : Identification of bioenergetic modulators adapted to the metabolic profile of the cancer cells (→Determination of the IC50 of metformin)

We brought the precision of the Bravo platform to the high throughput efficiency of the Agilent Seahorse XF96 Analyzer and CELLOMET’s know-how.

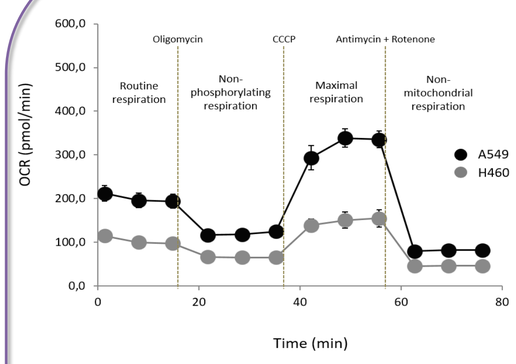


For each objective we seeded 30.000 cells of each of the two lung cancer cell lines (A549 and H460) in each well of a 96-well plate. Respiration was measured using the Agilent Seahorse XF96 Analyzer at the CELLOMET bioenergetic investigation in Bordeaux (France). A mitostress kit was used in the cartridge plate to measure basal, minimal and uncoupled respiration rate.

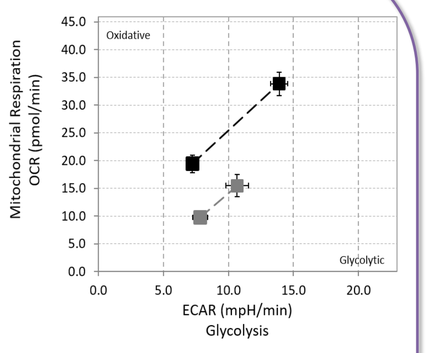
Z prim evaluation : The precision and reproducibility of our procedures can be evaluated by assessing the Z prim factor, which is a measure used for the GO/NO GO decision when performing high throughput screens of drugs or siRNA libraries. When precised, the cells were incubated with a cocktail of mitochondrial inhibitors for two hours. After this incubation time, a Seahorse test was performed at the CELLOMET facility.

IC50 of metformin test : The performance of the Bravo automate in the dilution of compounds was tested by the evaluation of the IC50 of metformin, a complex I inhibitor approved in the clinics for the treatment of diabetes. Metformin efficacy was tested on the cell lines of varying bioenergetics in co-treatment with doxorubicin, a valid chemotherapeutic drug (1µM). Different concentrations of metformin (0µM to 4mM) were tested, during 24h. To complete the results obtained on IC50 measurement with the Agilent Seahorse XF96 Analyzer, we performed a measure of respiratory chain complex I spectrophotometric activity using CELLOMET standardized protocol.

Objective 1 : Bioenergetic profiling of two lung cancer cell lines



Seahorse analysis allowed to **stratify** two cell lines according to their metabolism. A549 is oxidative and H460 is more glycolytic.



The coefficient of variation is a useful statistical index for comparing the degree of variation from one data series to another.
Interpretation : 0<CV<4,9% : excellent, 5%<CV<9,9%: very good 10%<CV<14,9%: good, 15%<CV<24,9%: acceptable, 25%<CV :marginal

coefficient of variation (CV)	A549	H460
Routine Respiration	6,6	8,1
Non phosphorylating respiration	4,6	3,2
Maximal respiration	9,3	7
Non-mitochondrial respiration	3,9	4,8

The coefficient of variation is very good in each cell line

→ Agilent Seahorse XF96 Analyzer can stratify cancer cells according to their bioenergetics

Objective 2 : Bravo platform versus human pipetting: Where’s the difference? (→Evaluation of Z prim factor.)

The Z’ factor is a statistic tool that permit to evaluate the precision of pipetting. The Z’ robust factor is used for the high- throughput screening assay.
Interpretation of Z’ factor : Z’ = 1 : Ideal; 0,5<Z’< 1 : an excellent assay; 0<Z’<0,5 : a marginal assay

A549	Bravo	Human
Z’	0,70	0,5
Z’ Robust	0,86	0,65

H460	Bravo	Human
Z’	0,73	0,22
Z’ Robust	0,89	0,12

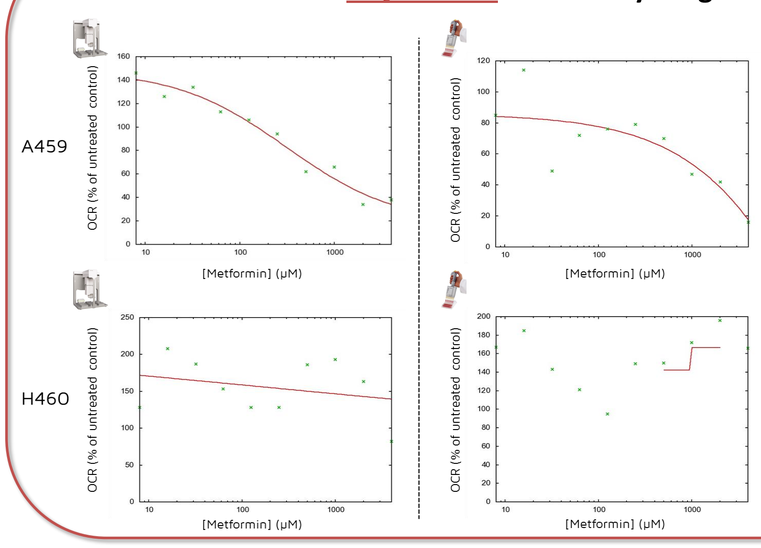
Z’ factor was calculated from the data of respiration using OXPHOS inhibitors as positive controls

→In A549 cells as in H460 cells, the Z’ factor and the Z’ robust factor was better (close to 1) with Bravo platform than human pipetting.

→ The Bravo platform has a much better precision of pipetting than a trained qualified engineer

CELLOMET Agilent Collaboration

Objective 3 : Secondary drug screening using a phenotypic read-out (mitochondrial respiration) and hit validation by targeted CELLOMET assay



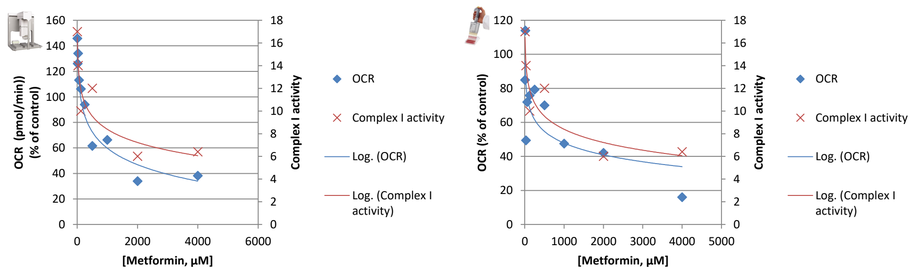
We hypothesized that metformin could alter specifically the oxidative lung cancer cells (A549) based on the metabolic profiling analysis (objective 1).

→In A549 cells with Bravo pipetting, the IC50 determination curve showed a sigmoid profile that could not be established with human pipetting.

No significant cancer-killing effect of metformin was found on H460 cells of glycolytic profile.

→The Bravo automate combined to Agilent Seahorse XF96 Analyzer allowed to find one drug, metformin, that specifically targets cancer cells with oxidative bioenergetic profile

MOA study by CELLOMET confirmed that Metfomin inhibits Complex I in A549 cells



→ With the Bravo platform the determination of the IC50 is more precise than human pipetting and IC50 determination more precise.

→ CELLOMET targeted enzymatic assay provide a MOA for metformin anti-cancer effect.

CONCLUSION

Combining the Bravo Automated Liquid Handling Platform and the Agilent Seahorse XF96 Analyzer provides an accurate **bioenergetic platform** with excellent characteristics in terms of reproducibility and efficiency. Such platform can be used to study cellular energetics in a large number of contexts and to discover drug or gene modulations that impact mitochondrial respiration and cellular glycolysis. The Seahorse-Bravo equipment was validated by CELLOMET experts and a proof-of-concept experiment presented here showed the differences between A549 and H460 cells as well as their sensitivity to mitochondrial respiration by metformin.

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