Advancing cancer cell biology with a physiologically relevant cell culture medium: Plasmax™

Application note

- Plasmax™ has been developed as a specialised cell culture media to improve the metabolic fidelity of *in vitro* cancer cell models
- As a complete ready-to-use medium, Plasmax™ comprises of over 50 components optimised to physiological concentrations found in human plasma.
- Plasmax™ mitigates the pseudohypoxic state seen with traditional culture media and effectively sustains cell proliferation to provide novel insights at the phenotypic, transcriptomic and metabolic levels.

Introduction

Traditional cell culture media were originally developed in the 1950s with a primary objective – to rapidly and successfully increase cell proliferation in an *in vitro* environment (Eagle, 1955). This was made possible by adding nutrients in excessive concentrations to avoid nutrient depletion and simultaneously promote cell growth. Such disproportionate nutrient composition, in comparison to *in vivo* conditions like human plasma, affects both phenotypic and genotypic behaviour of cells (Schug et al, 2015 and Tardito et al, 2015). Hence, usage of traditional media for cell culture can lead to unrepresentative *in vitro* conditions and variance between *in vitro* and *in vivo* cancer cell metabolism. This becomes particularly important in research relating to cancer cell biology and related metabolic pathways.

To address this challenge, the research team at the Beatson Institute for Cancer Research, Glasgow, UK, under the supervision of Dr. Saverio Tardito, an oncometabolism expert, developed a novel cell culture medium, Plasmax™, to study the cell metabolism in different tumour types. Plasmax™, was formulated by optimising over 50 components to physiological levels found in human plasma.

By reproducing the physiological cellular environment, Plasmax™ improves the robustness and fidelity of *in vitro* cancer cell models which helps in avoiding metabolic discrepancies between results obtained with *in vitro* cell culture and *in vivo* models. Further, using such a physiological media can also uncover novel biological findings within the cancer research space at the phenotypic, transcriptomic and metabolic levels.

Since its development, Plasmax™, has been successfully tested on a variety of primary and established cell lines, spheroids and 2D and 3D tumour models, which demonstrates its versatility and value as a specialised cell culture medium for researchers studying cancer cell biology.

Product overview: Plasmax™

Given its physiological relevance to *in vivo* conditions, Plasmax™ improves the fidelity and biological significance of *in vitro* cancer models.

- **Physiologically relevant**
  Plasmax™ is optimised to reflect the *in vivo* profiles of nutrients and metabolites found in human plasma, including essential and non-essential amino acids, amino acid derivatives, organic acids, and other polar metabolites.
- **Improves *in vitro* metabolic fidelity** Plasmax™ can better approximate the overall metabolic phenotype of tumours, with both 2D and 3D cells cultured in Plasmax™, better recapitulating the tumours' metabolic signatures.
- **Uncover role of trace elements**
  Cancer cells seeded at low densities in the absence of the trace element selenium are unable to form colonies in traditional media due to lipid peroxidation and ferroptosis. The growth-enabling trace elements in addition to vitamins and inorganic salts in Plasmax™, prevent ferroptosis-induced cell death, and promote colony growth.
- **Complete ready-to-use formulation**
  Plasmax™'s unique ready-to-use formula maintains its effectiveness throughout its shelf life with no effect on cell growth from aged Plasmax™. It is compatible across different cell types and *in vitro* cell based models.

Results

Chemically defined with components at physiological concentrations

The formulation of traditional media is limited to a select number of nutrients at supraphysiological levels. In DMEM-F12, for instance, glucose and glutamine alone provide >75% of the nutrient source. In contrast, these nutrient levels in Plasmax™ account for less than 50% of the total nutrient pool (Vande Voorde, et al. 2019). Important nutrients such as Choline, Pyruvate, Glucose, Glutamine, and Arginine, are present in historic media at concentrations exceeding by several folds those found in blood circulation (See Fig. 1a).

In addition, 35% of components found in Plasmax™ are absent in traditional growth media, but present in human plasma, making Plasmax™ a more physiologically relevant cell culture medium.

Nutrient exchange that better reflects the *in vivo* environment

The intracellular metabolite levels of cells cultured in Plasmax™ are proportional to the concentrations found in traditional media (see Fig. 1b-c).

The excessive nutrient levels in traditional media such as DMEM-F12 evoke compensatory metabolic shifts in cell cultures. For example, having excess arginine in culture media causes artefactual reversal of a reaction of the urea cycle, forcing the direct production of argininosuccinate from arginine (see Fig. 1d-e). In comparison, cells grown in Plasmax™ derived argininosuccinate from citrulline, as in the canonical urea cycle pathway (see Fig. 1f).

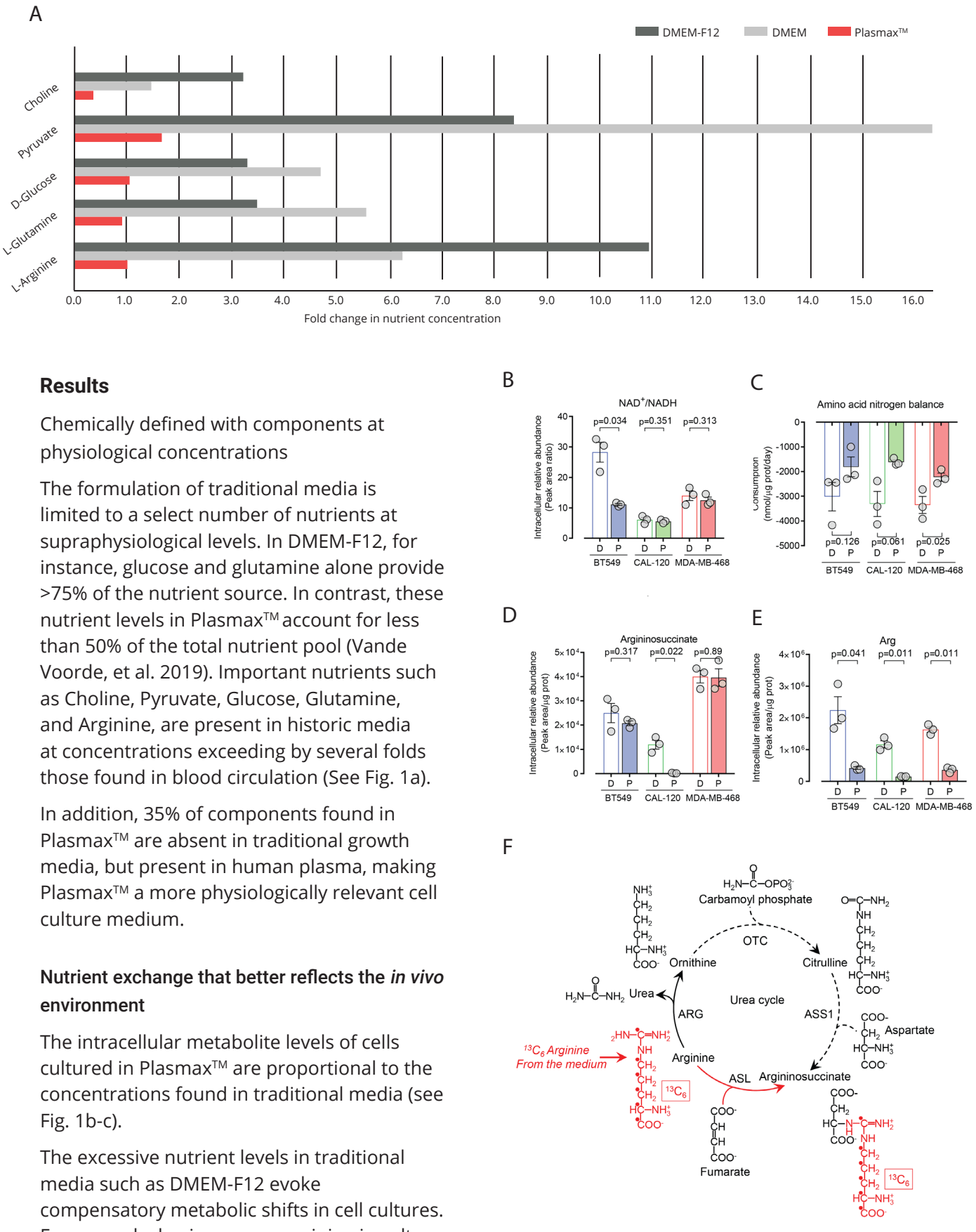

Fig.1. (A) Fold change in nutrient concentration relative to human plasma. Amended from Ackermann et. al., 2019. (B-E) Consumption of the amino acid bound nitrogen and intracellular abundance of metabolites and metabolite ratios in cells cultured in Plasmax™ and DMEM-F12. (Means ± SEM; n = 3). (D=DMEM-F12, P=Plasmax™). (F) Schematic representation of the urea cycle and expected labeling of argininosuccinate from $^{13}C_6$ arginine upon reversed ASL activity. (Vande Voorde et al., 2019).

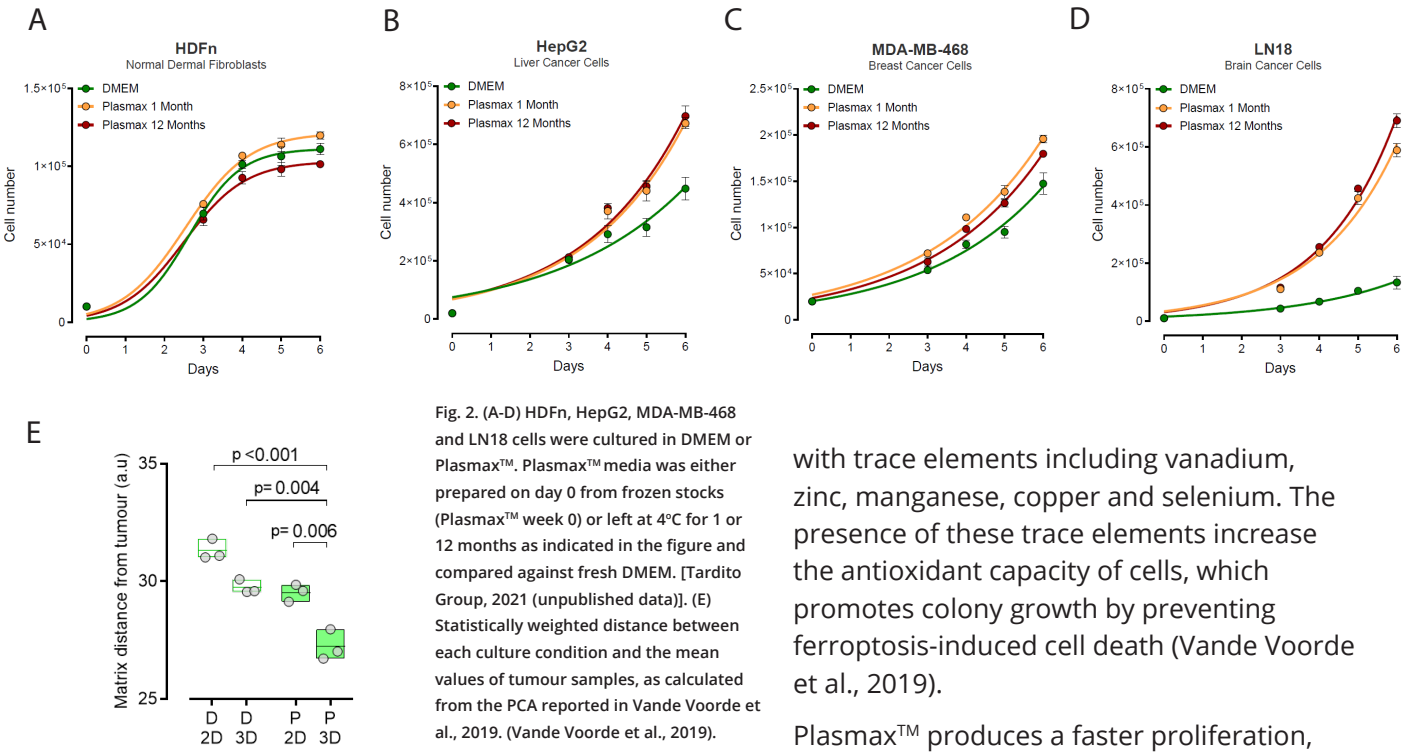

Fig. 2. (A-D) HDFn, HepG2, MDA-MB-468 and LN18 cells were cultured in DMEM or Plasmax™. Plasmax™ media was either prepared on day 0 from frozen stocks (Plasmax™ week 0) or left at 4°C for 1 or 12 months as indicated in the figure and compared against fresh DMEM. [Tardito Group, 2021 (unpublished data)]. (E) Statistically weighted distance between each culture condition and the mean values of tumour samples, as calculated from the PCA reported in Vande Voorde et al., 2019. (Vande Voorde et al., 2019).

Table 1. A selected list of cultured cell lines successfully validated for growth and viabiliy in Plasmax™ under standard conditions.

Cell lines grown in Plasmax™	Tissue of origin	Cell line status	Species
HepG2	Liver cancer	Established line	Human
HuH7	Liver cancer	Established line	Human
HuH6	Liver cancer	Established line	Human
BT549	Breast cancer	Established line	Human
MDA-MB-468	Breast cancer	Established line	Human
Cal120	Breast cancer	Established line	Human
A375	Melanoma	Established line	Human
Colo829	Melanoma	Established line	Human
LN18	Brain cancer	Established line	Human
Naive glioblastoma cell line	Brain cancer	Low passage lines	Human
Dermal fibroblasts	Epidermis	Primary	Human
Small intestine organoid	Small intestine	Primary	Mouse
Mammospheres	Mammary gland	Primary	Mouse
Mesenchimal stromal cell line	Bone marrow	Primary	Human
Embryonic stem cell line	Embryo	Primary	Human
Trophoblast stem cell line	Placenta	Primary	Human
A549	Lung cancer	Established line	Human
HCT116	Colon cancer	Established line	Human
SaOS2	Bone tumour	Established line	Human
HT1080	Fibrosarcoma	Established line	Human

A ready-to-use medium with growth-enhancing trace elements

Trace elements while essential for survival and proliferation, are often missing from traditional cell culture media and have to be supplemented before use. Plasmax™ is uniquely formulated with trace elements including vanadium, zinc, manganese, copper and selenium. The presence of these trace elements increase the antioxidant capacity of cells, which promotes colony growth by preventing ferroptosis-induced cell death (Vande Voorde et al., 2019).

Plasmax™ produces a faster proliferation, even when aged up to 12 months, in comparison with DMEM when both are supplemented with 2.5% foetal bovine serum

Improves the metabolic fidelity of *in vitro* cancer models

Cancer cell lines grown in Plasmax™ have metabolic profiles that better recapitulate those of orthotopic tumour xenografts (Vande Voorde et al., 2019).

Over 100 metabolite levels were analysed in CAL-120 cancer cells grown in Plasmax™, DMEM-F12, or in tumour xenografts. A principal component analysis revealed that 2D or 3D cell cultures grown in Plasmax™ produced metabolite profiles closer to those of tumour xenografts than those grown in DMEM-F12 (see Fig. 2e).

Validated across a broad range of cell lines

Over the last 24 months, Plasmax™ has been successfully validated across primary cells of different tissue, species, and experimental conditions, (see Table 1), and is suitable for both primary and established cell lines.

Additional cell lines are successfully cultured using Plasmax™ on a regular basis, which makes Table 1 a running list of validated cell lines. Plasmax™ is anticipated to work across a broad range of cancer cell culture models.

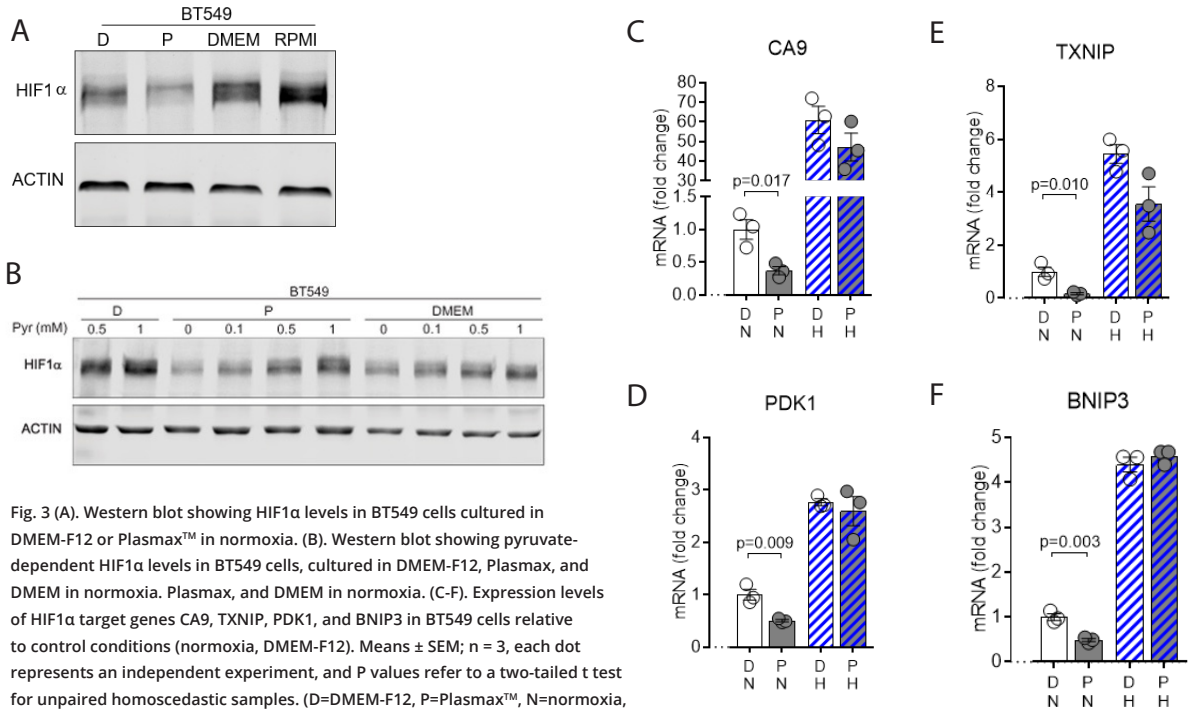

Fig. 3 (A). Western blot showing HIF1α levels in BT549 cells cultured in DMEM-F12 or Plasmax™ in normoxia. (B). Western blot showing pyruvate-dependent HIF1α levels in BT549 cells, cultured in DMEM-F12, Plasmax, and DMEM in normoxia. Plasmax, and DMEM in normoxia. (C-F). Expression levels of HIF1α target genes CA9, TXNIP, PDK1, and BNIP3 in BT549 cells relative to control conditions (normoxia, DMEM-F12). Means ± SEM; n = 3, each dot represents an independent experiment, and P values refer to a two-tailed t test for unpaired homoscedastic samples. (D=DMEM-F12, P=Plasmax™, N=normoxia, H=hypoxia) (Vande Voorde et al., 2019).

Mitigates the pseudohypoxic state in cultured cells

Analyses in the BT549 breast cancer cells revealed that, under normoxic conditions (21%O_2), cells grown in DMEM-F12 exhibited a hypoxia-like transcriptomic signature (see Fig. 3). This included the increased expression of hypoxia-inducible factor 1-alpha (HIF1α), and several of its targets including CA9, TXNIP, PDK1, and BNIP3 (see Fig. 3b-e) (Vande Voorde et al., 2019).

This hypoxia-inducible factor 1-alpha stabilisation results from excessively high pyruvate concentration (e.g. 1mM), which is commonly found in traditional media. This hypoxia-like effect was not observed in cells grown in Plasmax™, which contains physiological levels of pyruvate (0.1mM).

Conclusion

Plasmax™ is a physiologically relevant cell culture medium that closely resembles the metabolic and nutritional profile of human plasma. Unlike traditional media designed to supply excessive levels of a few nutrients, Plasmax™ provides unmatched metabolic fidelity in a unique ready-to-use formula.

Plasmax™ has been validated in a broad range of cell types and experimental conditions. It effectively sustains the growth of cells seeded at low densities, provides novel insights into the role of trace elements in cancer cell biology, and prevents artefacts forced by the inequitable nutritional composition of traditional media.

A partnership between the Cancer Research UK Beatson Institute and CancerTools.org, part of Cancer Research UK's Commercial Partnerships team, was established to scale up the production, commercialisation and distribution of Plasmax™, ensuring the cell culture medium was made easily accessible to other researchers worldwide.

About CancerTools.org

CancerTools.org mission is to create an embracing non-profit, global community of researchers, institutes and societies, to make research tools available from and to cancer researchers around the world, to accelerate cancer discoveries.

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