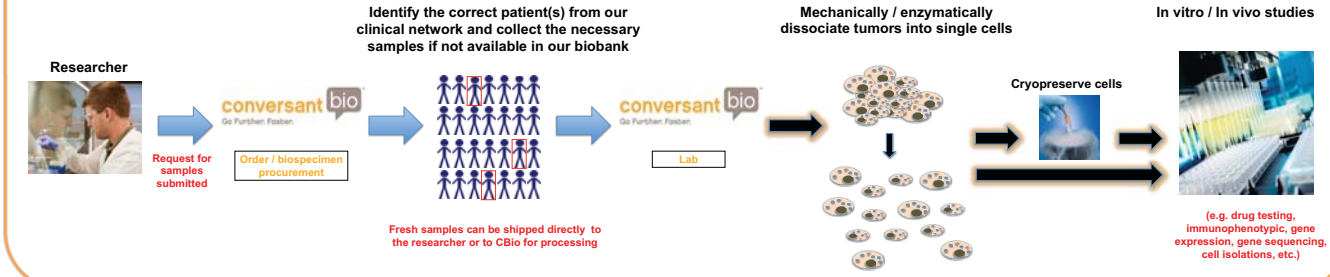
Development of a Standardized Drug-Testing Platform Using Human Primary Tumor Samples

Chris Kidd, Oke Ebenezer, Crystal Phillips, Kyle Lewter, Allen MacDonald, Dawn Adkins, Will Taylor, Obdulio Piloto. Conversant Biologics Inc., Huntsville, AL

ABSTRACT:

In the past few years, various groups have demonstrated the role of tumor heterogeneity and the importance of using human primary cells, over established cell lines, to understand cell-cell, cell-extracellular matrix (ECM) and cell-drug interactions. In addition, access to an array of highly annotated, viable human primary samples that are processed in a standardized fashion is often the limiting factor for many academic and industry researchers. Building on these observations, we developed a standardized platform for collecting, processing and testing human primary samples (i.e. solid tumors, normal adjacent tissue, normal or diseased bone marrow / blood) from our network of clinical sites. Tumors were collected immediately after surgical resections and transported in HypoThermosol solution (BioLife Solutions, Inc). Using our optimized protocols, these tumors were enzymatically and mechanically dissociated into single cells and cryopreserved in CryoStor media (BioLife Solutions, Inc). The post-thaw viability of over 100 tumor samples, representing all major tumor types, was found to be 70-100% by Trypan blue exclusion. Flow cytometric characterization of these samples revealed that most samples contained 30-80% EpCAM+ (CD326+) cells, with colorectal tumors possessing the greatest percentage of EpCAM+ cells (60-80%). Further studies demonstrated that most human primary tumors contain <20% hematopoietic cells (CD45+/CD31-), <10% endothelial cells (CD31+/CD45-) and <10% putative cancer stem cells (CD326+/CD133+). A select group of patient samples with a high percentage of EpCAM+ cells were plated onto tissue-culture (TC) treated plates, BD Matrigel coated plates and embedded into BD Matrigel (3D assay) to assess their viability / proliferation over 6 days and their response to drug treatment. Most samples remained viable or proliferated during the 6-day study, yet some differences were observed between TC-treated and BD Matrigel coated plates. As expected, cells from different human primary tumors responded differently to a 3-day treatment with the tool compound staurosporine (8-point dose response). These studies also revealed that large assay windows (typically >50) are routinely observed using human primary cells. This assay is also reproducible, since similar dose-response curves were observed when human primary tumor cells were re-tested. In one example, cells from an ovarian metastatic tumor were completely unresponsive to staurosporine treatment when embedded within BD Matrigel, but responsive when cultured on TC treated and BD Matrigel coated plates. In summary, our data highlights our efforts to optimize and standardize the collection and processing of human primary samples and the development of a drug-testing platform that is available to the research community. An array of different in vitro (i.e. tumor spheres, transwells, monocultures) and in vivo assay formats is currently being explored with our human primary samples, both from solid tumors and hematopoietic malignancies.

Overview of Standardized Platform

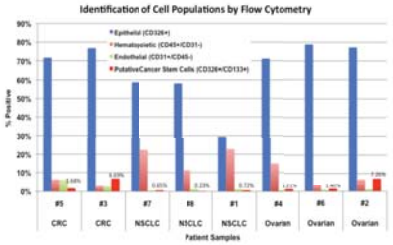


Characterization of dissociated solid tumor cells

Flow Cytometric Analysis of 25 Post-thawed Human Primary Solid Tumor Specimens

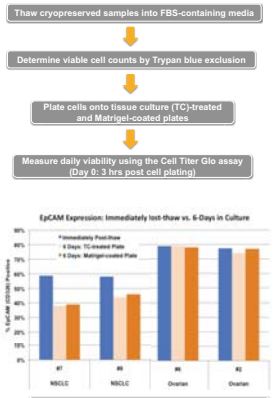
	Defined as:	Lung	Ovarian	Colorectal
Epithelial	CD326+	10 - 60%	12 - 80%	20 - 80%
Hematopoietic	CD45+/CD31-	6 - 23%	3 - 30%	1 - 10%
Endothelial	CD31+/CD45-	0.3 - 1.5%	0.2 - 5%	2 - 10%
Putative cancer stem cell	CD326+/CD133+	0.1 - 4%	0.5 - 7%	0.1 - 12%

Disease	Patient ID	Post-thaw Viability
CRC - Adenocarcinoma	#5	100%
CRC - Undefined	#3	100%
NSCLC – Squamous Cell Carcinoma	#7	100%
NSCLC - Undefined	#8	72.40%
NSCLC – Adenocarcinoma	#1	100%
Ovarian - Adenocarcinoma	#4	100%
Ovarian - Adenocarcinoma (metastatic tumor)	#6	100%
Ovarian - Undefined	#2	83.30%



Post-thaw viability for >100 human primary tumor samples: >70%

6-Day Viability & Flow Cytometric Study



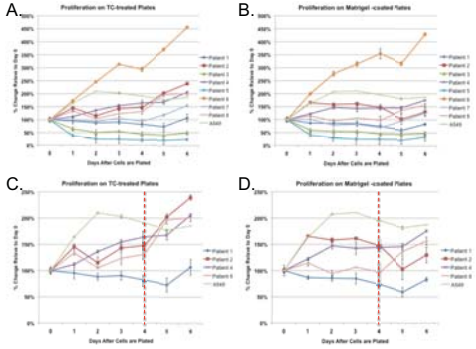
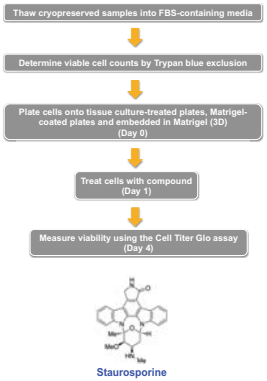


Figure 2. The percentage of EpCAM+ cells immediately post-thaw and following 6-days in culture by flow.

* Error bars denote %CV

Figure 1. 6-day viability study examining 8 primary solid tumors and 1 cell line (A549). Daily viability results for cells plates on (A) tissue-culture treated plastic and (B) Matrigel coated plates. (C) & (D) Select samples showing altered growth patterns when plated on tissue-culture treated plastic compared to Matrigel coated plates. Viability was measure with Cell Titer Glo.

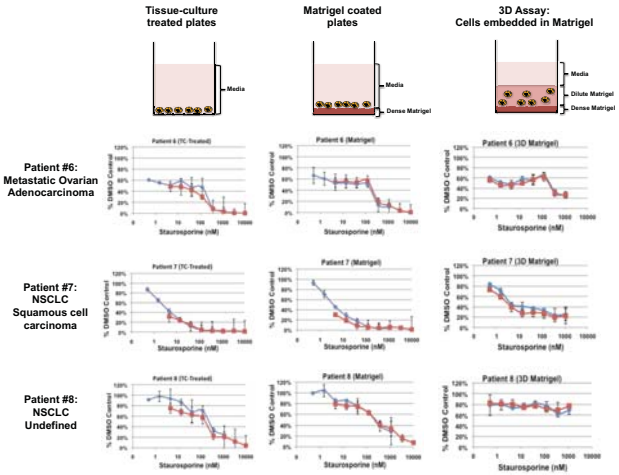
Examples of in vitro compound testing



Staurosporine

- Prototypical ATP-competitive kinase inhibitor
- Binds to serine / threonine and tyrosine kinases
- Non-kinase selective inhibitor
- Potent inhibitor of PKC
- Low [nM] result in cell-cycle arrest (cytostatic)[1]
- Higher concentrations induce apoptosis[2]

[1]McGahren-Murray, Mollianne, et al., Cancer Research, 2006
[2]Belmokhtar, Chafika Ahmed, et al., Oncogene, 2001



* Error bars denote %CV

NOTE: observed signal-to-noise ratios of 10-400 (luminescence from DMSO control wells / 20uM treated wells on the same plate)

Figure 3. Cells from three human primary solid tumor specimens were plated onto tissue culture treated plates, Matrigel coated plates and embedded in Matrigel (3D assay). The following day, cells were treated with staurosporine for 3 days. Viability was measure with Cell Titer Glo. The identical study was repeated ~1 month later by a different person and the blue and red curves are the results from each study.

Summary:

- We developed an optimized, standardized platform for ordering, collecting, shipping, processing, cryopreserving and analyzing human primary solid tumors (similar platform was developed for blood / bone marrow malignancies… not shown)
- Tested human primary solid tumors contain 10-80% EpCAM+ epithelial cells, 1-30% hematopoietic cells, 0.2-10% endothelial cells and 0.1-12% putative cancer stem cells.
- The post-thaw viability of dissociated solid tumor cells is typically >70%.
- Most tested human primary solid tumor cells remain viable for 6 days in culture and some samples show signs of proliferation.
- 3 of 8 tested patient samples demonstrated altered proliferation when cells were plated on tissue culture treated plastic compared to Matrigel coated plates; this change in cell growth was most evident from day 4-6.
- The percentage of EpCAM+ cells on tested lung and ovarian cancer specimens decreases by 0-20% following 6 days in culture.
- Cells cultured for 4 days and treated with staurosporine for 3 days, show similar drug responses when cells are plated on tissue culture treated plastic or Matrigel coated plates. When the same study was carried out with cells embedded in Matrigel (3D assay), 2 of 3 samples showed slight drug resistance at higher doses while 1 of 3 samples showed complete drug resistance at all doses.