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Note Article

CM1106 STEMCHEM: Chemical Approaches to Targeting Drug Resistance Cancer Stem Cells

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Abstract. STEMCHEM is a COST action aiming to target causes of drug resistance in cancer stem cells. Cancer stem cells are cells which are believed to be responsible for the larger part of the regenerative capacity of cancers. They are also thought to be similar to adult stem cells in that they do not proliferate most of the time and are thus resistant to many kinds of chemotherapy. The action brings together labs around Europe in both biological and chemical fields to work together in this regard. Biologists targeting individual stem-cell related molecules as well as stem cell phenotypes (like the undifferentiated state), test chemicals from numerous labs for activity in high throughput screens, with the aim of identifying new drug targets. This COST action, like most others, offers opportunities for Malta, both in a general way and also particularly for a small country with small labs.

Keywords: Stem cells; Cancer; Differentiation; COST; drug resistance

COST (European Cooperation in Science and Technology) is one of the longest running European Scientific programmes, which allows for better coordination of nationally funded research at a European level.

Unlike the FP7/Horizon 2020 instruments which work in a top-down manner with calls being issued by the Commission as to what areas of research it is interested to fund, COST works in a bottom up or grass-roots approach. Any group of labs or interest groups from a few different countries can get together, start up a COST action, after it is vetted by the COST central administration in Brussels and then open it up to other partners to join in. This information is usually passed on the national contact point organisation (in Malta the MALTA COUNCIL FOR SCIENCE AND TECHNOLOGY), who informs interested potential participants on a regular basis of the newest set of COST actions to be set up.

For a small country like Malta, with small labs and minimal funding, where entry into the big consortia characteristic of the FP programmes has always been difficult (and I speak from experience here), COST allows the development of important scientific contacts, which may later lead to participation in such collaborations. It also allows increased scientific exposure of research done in our laboratories.

The University of Malta's Anatomy Department through a number of graduate students under Pierre Schembri-Wismayer's supervision has been working for some time on inducing differentiation of leukaemia cell lines using a variety of natural extracts, some of which have been published (Agius Anastasi, Cassar & Schembri-Wismayer, 2012) and others of which not yet due to the possibility of developing intellectual property for the University. The research uses the HL60 leukaemia cell line as an initial screen since it allows differentiation into both monocytic and granulocytic cell types.

When reviewing newer COST actions, Prof. Janet Mifsud, COST coordinator in Malta brought to our attention an action entitled "Chemical Approaches to Targeting Drug Resistance in Cancer Stem Cells" in December 2011. This seemed interesting so I, (Pierre Schembri-Wismayer) approached Prof. Mifsud about joining and was elected a member on the managerial board of the Action, representing Malta. I attended the

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kick-off meeting in Brussels, with the rest of the management committee since we got involved early on. The meetings of the management committee (which tend to be combined with workshops in this Action in order to allow more to get done at once) allow the regulation of the budget and of how the action is set to work.

Cancer stem cells (CSC) are a subpopulation of cells within tumours that exhibit enhanced tumor-initiating attributes and are a major contributing factor to current cancer therapy failure. The CSC phenotypic state comprises distinct molecular and functional differences that underpin resistance to current treatments and the unique ability spread and to seed new tumors throughout the body. This insight of this particular subpopulation of cells and its capability of repopulating tumours where most cells have been killed by conventional therapy, necessitates an entirely new approach to cancer drug development. This action aims to unite expertise in rational drug design and medicinal chemistry with biomedical investigators dedicated to understanding the mechanisms governing drug resistance in cancer stem cells. Thus it aims to develop effective methods for identifying novel compounds and drug candidates that target these drug-resistant cancer cells. One such way in fact would be to cause these cancer stem cells to differentiate into less stem-like cancer cells so that they can then be killed by more standard chemotherapy. The rationale and basis behind the action are reviewed in more detail in a recent publication from the consortium (Sotiropoulou, Christodoulou, Silvani, Herold-Mende & Passarella, 2014).

During the first meeting in Brussels we also chose a number of important positions such as the Chair (Prof. Daniele Passarella, a chemist from University of Milan, Italy who had initiated the action) and Vice Chair (Prof. Marija Balic, a medical doctor and biologist from Austria) as well as a number of important positions relating to specific instruments of the actions, (such as Dr Gabriela Almeida from Portugal who is in charge of short term scientific missions - STSMs - more about these later).

It was decided for example that members were divided into three working groups, one of chemists, one of biologists and one of pharmaceutical and medicinal chemists, including specialists in computational prediction of drug-target interactions. Our lab joined working group one, primarily for biologists. However, even within this group, there were numerous variations, which enriched the meetings since attending the various workshops not only allowed sharing of our own expertise but learning from others.

Amongst the biological experts were clinicians developing novel treatments for brain cancers, biologists developing *in vitro* models for various cancers, scientists developing *in vitro* systems of testing different well known stem-cell related candidate targets like Notch and Hedgehog, those screening for epigenetic modifiers of the stem cell phenotype and others like ourselves involved in phenotypic screening.

Chemists also hailed from different branches, including synthetic chemistry, producing steroids, retinoids and other potential drugs, pharmaceutical chemistry, *in silico* screening and development of chemical libraries.

These three groups worked within themselves, each setting up different workshops at the different group meetings. They also set up collaborative activities, such as the development of a chemical database of different kinds of agents for testing, from the chemists. However, it later became clear that the best work and publications would result from the development of cross-speciality collaborations where biologists tested new chemicals in their various test systems. In fact the three work-group system whilst still a functional grouping, became less significant as time went by. Numerous collaborations have been developed over the period of the COST action (although it is still ongoing) resulting in various publications (Madeira et al., 2014; Christodoulou et al., 2013; Majchrzak et al., 2013; Porcile et al., 2014).

Once the action was established, the first workshop was held in Milan in July 2012 and two members from our research group, Dr Pierre Schembri-Wismayer and Dr Krystle Blaire Theuma attended. The meeting was very well organised and showed many different approaches to tackling stem cells in Cancer, including both the targeting of specific stem cell-related molecules using chemical approaches and the wider search of natural or synthesised products for anti-stem cell activity using a particular biological model system.

One of the main points about such meetings is that apart from the consortium members themselves contributing, external speakers (usually experts in the field who can contribute a new view point or angle to ongoing research) are invited (and funded) to attend the different meetings.

We presented some research work, using natural extracts for leukaemia differentiation. An oral presentation presented by PSW was entitled "Insect cell-extract induces increased expression of differentiation markers in HL60 leukaemia cells" whilst Dr Theuma (also a managerial committee member in the STEMCHEM consortium) presented a poster entitled "Combination of DNA modifying agents and differentiation inducers can enhance differentiation in HL60 leukaemia cells", which was work she had done in collaboration with Ms Analisse Cassar (Fig. 1).

The advantage of this was that since we were working using phenotypic screening (looking for a biological effect which could lead to candidate compounds for drug development and since we (mostly A. Cassar) has de-

Figure 1: Dr Schembri-Wismayer and Dr Theuma presenting their poster and discussing with Prof. Navakauskiene.

veloped this phenotypic screen into a relatively high throughput system, many chemists in particular, were interested in talking to us to assess their compounds. The fact that the group's area of expertise is also the less commonly targeted mechanism of differentiation therapy rather than cytotoxicity also increased interest.

Basically, we spectrophotometrically assess a leukocyte differentiation-related enzymatic activity (NBTZ). This is then divided by a mitochondrial activity which acts as a surrogate for cell number giving us and indication of the average differentiation marker per cell (Fig. 2).

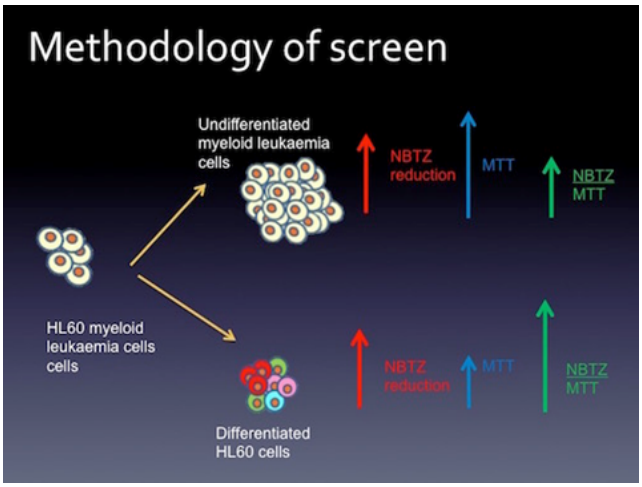

Figure 2: Schematic showing rationale behind screening tests.

At a second workshop meeting in Porto in February 2013, Analisse Cassar, another graduate student from the same lab presented her paper "Effects of Insect Conditioned Medium in Combination with Chromatin-Modifying Agents on the Terminal Differentiation of Leukaemia". She was given a lot of good feedback and I (PSW) received glowing reports of her very good presentation of very interesting work from other very authoritative STEMCHEM colleagues.

Analisse presented this work in the same session as a colleague form Lithuania, Ruta Navakauskiene from Vilnius University, who presented complimentary and similar work entitled "Effects of HDACI, HMTI and HMTI in combination with retinoic acid on granulocytic differentiation of human promyelocytic leukemia cells". Our lab and that of Prof. Navakauskiene have often found that we are working on similar areas so sharing our expertise and skills in STEMCHEM has been useful in turning us from primarily competitors to collaborators.

In fact, another post-graduate student in the lab, Mr Sherif Suleiman, supervised by Dr Jean Calleja Agius, the present head of the Anatomy department will hopefully be benefiting form a short term scientific mission to Prof. Navakauskiene's labs in Lithuania, where he will be learning how to test for different chromatin-modifying agents using molecular biology techniques not presently in common use in our own labs. We on the other hand have benefited from colleagues in Europe visiting our lab, from Greece (officially funded as an STSM) , Ireland and Serbia, through the STEMCHEM consortium.

This is in fact the aim of a short terms scientific mission. This is funded as part of the action and involved up to €300 for travel and €60–€90 as a daily allowance up to a maximum of €2500 for a period up to 3 months and in the case of early stage researchers (within 8 years of a PhD), even up to €3500 for longer periods.

I should at this point make a little note as to the various benefits of COST actions for our little nation. Many countries have two management committee members from two different labs. In our consortium, the management committee members are always funded in whatever conference or visit is organised, except when specific meetings for young scientists for example are organized when there is no management committee meeting . When possible, the Action also funds 1-2 participants from each other member (i.e. each lab or university involved in the action, since of course these may involve numerous labs from the same country). Since it is uncommon that too many researchers are involved in a particular area, in Malta and since one is allowed to have alternative management committee members, should one be able to attend, then it allows good funding locally for research staff, and even students to have the opportunity to present their work on a much wider stage.

In fact following the different talks by the group, collaborations initially started as contacts from individual labs who asked us to send certain chemicals for testing.

One of the earliest of these was Dr Danijel Kikelj who sent 12 chemicals for differentiation testing. Later Nadine Martinet (initially an invited speaker from INSERM in Nice in France and later a member of the consortium) asked all chemistry labs to contribute towards a large central chemical database which she administered and this was made available to all the various biology labs. As a result, we ended up screening over 600 chemicals (and still have more to work on) from numerous labs around Europe. Due to the large number of chemicals needing work-up and due to the limited hands on deck in terms of graduate students, more than 30 undergraduate students participated in this research, also offering undergraduate students (some of which are shown in Fig. 4) an opportunity to understand the research component of the University of Malta and contribute to international science. More than 80 very interesting hits (which cause leukaemia differentiation to different degrees) have been identified and many of these are being followed up.

The first 400 or so chemicals screened were presented in a fourth meeting in Budapest in March 2014 (no one from Malta attended the third meeting in Warsaw) and again this garnered a lot of attention with various labs interested in continuing collaborations. Some of the chemicals presented are indicated in Fig. 3 where different novel synthesised chemicals (named only by their catalogue number) are shown to be active in the screening method developed in our lab, again compared to positive and negative controls. The more active of these chemicals are then assessed by morphology – i.e. the effects of differentiation are assessed visually. Should this also be interesting, further testing can be done by means of flow cytometry (Fig. 5), locally to confirm the expression of differentiated chemistry markers. In fact this has resulted in students and post-docs from labs in Ireland and Serbia visiting our labs and more should be coming in the near future, from Greece and possibly elsewhere.

Figure 4: The students from the preclinical medical years who performed the bulk of the work in testing the first 400 chemicals on the STEMCHEM database.

Some of the chemicals from these labs have already shown activity in 2 or more assays and will be followed up for publication. The 80 or so hits from the first four hundred screen and all the new hits from the last 200 chemicals will also be assessed with morphology and flow cytometry and followed up accordingly with the

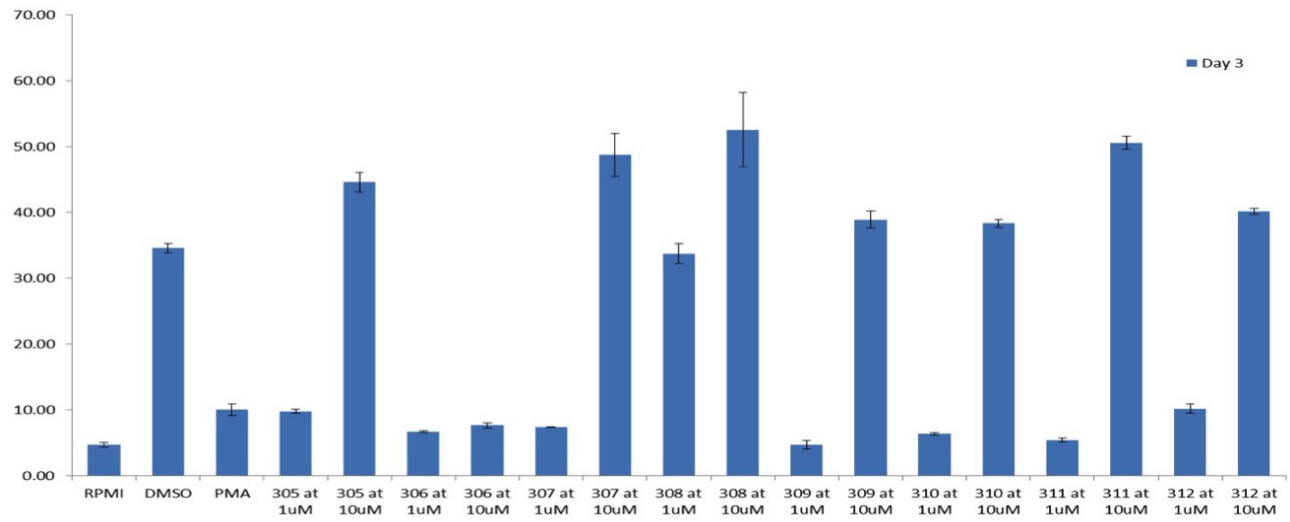

Figure 3: Some results out of the first 400 chemicals in the STEMCHEM chemical library, tested in the summer of 2013. Results show an index of differentiation created by dividing a leukocyte differentiation marker (NBTZ reduction function) by an indicator of cellular activity and thus of cell number (MTT activity). Results are an average of 3 replicates at each of two concentrations 1 and 10 mM. The first three columns show the results of negative control (normal culture medium) as well as two positive controls which however cannot be used in the clinic due to toxicity, PMA and DMSO.

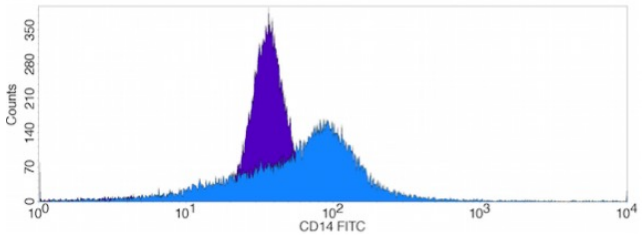

Figure 5: Changes in the expression of CD14 marker (monocytic differentiation) between untreated (violet) and treated (blue) HL60 populations.

chemists who produced them.

The common way that this is done is that the chemists make similar molecules to the hits and then these are once again screened to see which variations are even more active. The most active of these agents can then be tested on numerous leukaemia cell lines (we usually test 4 rather different myeloid leukaemias initially) and then can even be tested on primary leukaemia blasts from patients to see if the effect is also possible on patient cancers rather than the more artificial cell lines. All this information enhances the possibility of developing effective drugs for inducing differentiation, a rather novel kind of therapy for cancers, started a number of years ago with all-trans retinoic acid, which converted the normally fatal Acute promyelocytic leukaemia into a manageable disease where more than 90% of patients are cured nowadays.

Expanding this interesting branch of cancer therapy is one of the main interests of our lab at the Anatomy department and STEMCHEM has given us the capability to open up our expertise to collaborations all across Europe and to many more possible candidate drugs, apart from our own in-house derived extracts.

Another development in our own lab is the indication that many of these chemicals may also work on other solid tumours apart from leukaemias, especially the serious solid tumours of childhood. There is already evidence that all trans retinoic acid works to some extent on brain and bone tumours (Choschzick et al., 2014; Yang et al., 2012) but so do some of the extracts and drugs we are testing. In fact our preliminary work on osteosarcomas (bone tumours) was presented by another graduate student, Mr Sherif Suleiman (Fig. 6) at the last meeting of the Action in October in Tenerife where a good group of Action members enjoyed a meeting at one of Europe's more exotic destinations (Fig. 7).

Overall, COST actions offer extra funding for cash-strapped local research groups to travel, and train, and more importantly the opportunity to share data, discuss in a wider pan-European forum and set up collaborations which allow one to access larger sources of funding such as Horizon 2020 or Innovative Medicines

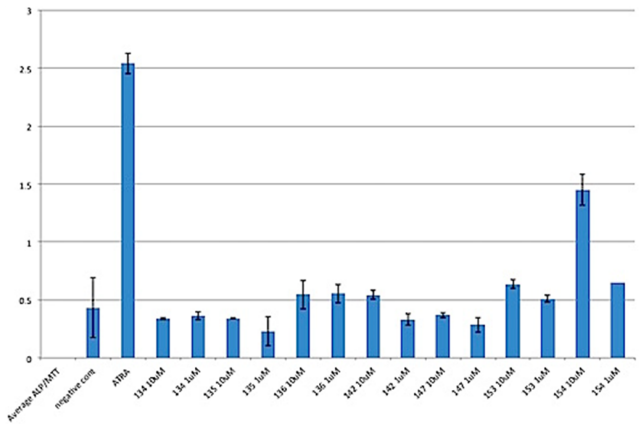

Figure 6: Changes in Alkaline phosphatase expression divided by indicator of total cell number assessing differentiation related changes in osteosarcoma.

Figure 7: The poster of the last action workshop meeting in Tenerife.

Initiative 2 funding. Importantly for our small country, they also provide an opportunity for students to travel and present their work. This was my first experience in COST but it will definitely not be my last.

Acknowledgments

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