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Keynote Lectures

Forecasting limits in personalised medicine*

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Resum. La medicina personalitzada significa trobar el tractament adequat per al pacient adequat en el moment adequat. Per bé que aquesta és una idea molt global i molt simple, és molt difícil d'aconseguir. Fer-ho requereix un esforç conjunt dels professionals de tots els àmbits: científics, polítics, advocats, economistes, etcètera, així com de sinergies entre investigadors, companyies farmacèutiques i biotecnològiques, i entre el sector públic i el privat. En la medicina personalitzada del càncer, un tumor s'analitza segons la genòmica, genètica, epigenòmica, característiques cel·lulars i bioquímiques que té, i se cerca la baula més feble per a millorar la probabilitat que el pacient respongui bé a la teràpia escollida. Els nombrosos avantatges d'aquest enfocament fan que sigui crucial per a donar suport a la investigació que conduirà al descobriment de nous biomarcadors, capaços de predir la sensibilitat d'un pacient als medicaments, per al seu ús en la medicina personalitzada.

Paraules clau: medicina personalitzada · epigenètica · epigenòmica · càncer · metilació del DNA · biomarcadors · *MGMT* · *BRCA1*

Summary. Personalised medicine means finding the right treatment for the right patient at the right time. Although this is a very global, very simple idea, it is nonetheless very difficult to achieve. To do so requires a concerted effort from professionals in all fields: scientists, politicians, lawyers, economists, etc., as well as synergies between researchers, pharmaceutical and biotech companies, and between the public and private sectors. In personalised cancer medicine, a tumour is analysed according to its genomics, genetics, epigenetics, epigenomics, cellular features, and biochemistry, and searches for its weakest link in order to improve the likelihood that the patient will respond to the therapy chosen accordingly. The numerous advantages of this approach make it crucial to support the research that will lead to the discovery of new biomarkers, capable of predicting a patient's sensitivity to drugs, for their use in personalised medicine.

Keywords: personalised medicine · epigenetics · epigenomics · cancer · DNA methylation · biomarkers · *MGMT* · *BRCA1*

Every year, 33,700 new cases of cancer are diagnosed in Catalonia; and one in two men and one in three women will develop cancer in their lifetime. Consequently, cancer is a very important health issue with serious financial implications. In the period 2001–2007, the number of cancer-associated deaths decreased by only 1.3–1.4 % and the 5-year survival rates have not been optimistic either: 51 % for men and 63 % for women. The slightly higher rate for women mainly reflects the fact that survival rates for breast cancer patients are around 83 % because of good management of this type of cancer, which is often detected at very early

stages. But there are also tumours for which the outcome of affected patients is dismal, such as small-cell lung cancer (5-year survival rate of 14 %).

Why has there been much less progress in treating lung and pancreatic cancers than breast cancer? Are there differences in the biology of these tumours? Is it only because of the earlier detection of breast cancer? We are not going to be able to get answers to these questions by random chance, nor is a single discovery in a small lab likely to cure cancer. Instead, a sustained effort is needed over many years, aided by the European Union in bringing together the best scientists around the world to tackle the problem.

If we look at different types of cancer in Catalonia (2003–2007, Fig. 1), we observe that the incidence of cancer is very similar to that in the rest of Europe: in men, prostate, lung and colon cancers are the most common malignancies. In women, breast cancer is the most frequent, but lung cancer is increasing, as women started smoking later such that only now is the peak of what is expected to be a massive incidence of lung cancer occurring.

	Cases, n	(%)			Cases, n	(%)
Prostate	4258	21.3		Breast	3907	28.6
Lung	3021	15.1		Colon and Rectum	2088	15.3
Colon and Rectum	3007	15.0		Uterus	734	5.4
Bladder	2238	11.2		Lung	527	3.9
Oral cavity and pharynx	788	3.9		Non-Hodgkin lynphoma	503	3.7
Stomach	669	3.3		Ovary	465	3.3
Non-Hodgkin lymphoma	605	3.0		Stomach	427	3.1
Liver	560	2.8		Bladder	420	3.1
Leukaemia	499	2.5		Luekaemia	413	3.0
Larynx	493	2.5		Pancreas	379	2.8

Fig. 1. Cancer incidence in Catalonia (2003–2007): The ten most common tumour types.

Personalised medicine in the treatment of cancer

What is the role of personalised medicine in the treatment of cancer? It is finding the right treatment for the right patient at the right time. Although this is a very global, very simple idea, it is nonetheless very difficult to achieve. To do so requires a concerted effort from professionals in all fields: scientists, politicians, lawyers, economists, etc. Personalised medicine is like finding the Achilles heel, the weakest point, in the disease process. In conventional therapy, if a person has a tumour, the oncologist or the pathologist will analyze a biopsy under the optical microscope.

But if the patient's tumour exhibits all the features of another patient's tumour, the same treatment will be administered for the same clinical stage, although the two patients may completely differ in their therapeutic responses. This is because the cellular and molecular repertoires of the tumours may be completely distinct, reflecting different genetic alterations. Personalised medicine, by contrast, considers a tumour according to its genomics, genetics, epigenetics, epigenomics, cellular features, and biochemistry, and searches for its weakest link in any one in order to improve the likelihood that the patient will respond to the therapy chosen accordingly.

There are many advantages to this approach, but first and foremost is the fact that patients receive the right treatment for the right tumour; second, the use of ineffectual therapies is avoided, which reduces healthcare costs and spares non-responding patients unnecessary toxicity while improving his or her quality of life.

"One dumb tumour is smarter than 100 oncologists"

This anonymous sentence appeared on the web a few years ago, but unfortunately it remains true today. The combined efforts of 100 oncologists cannot cure 80–90 % of lung tumours. Cancer is a complex disease that requires intense research efforts and investment. But there is light at the end of the tunnel. If we look at the genetics of cancer, what we have is a handful

of biomarkers that predict which drug the patient's tumour should be more sensitive to. Moreover, there may be five/six genetic mutations targeted by five/six approved drugs to which patients are likely to respond. But this is just the tip of the iceberg and much more research is needed before a complete therapeutic picture is obtained for each and every patient.

Our lab, like many labs around the world, is studying another aspect of cancer: epigenetics. Epigenetics considers DNA as a part of the story, but not the whole story. For example, all our cells contain the same DNA, but neurons look very different from muscle cells. This is because different chemical markers regulate the expression of genes, including those responsible for switching-on and switching-off other genes. These markers may consist of DNA-methylation, histomodifications, etc. and they are responsible for producing different cellular phenotypes. Thus, in monozygotic (identical) twins, even though their DNA is the same, one twin may develop breast cancer at age 25 and the other at age 65, because of epigenetic differences. In one twin the gene in question may be methylated, and in the other unmethylated [7].

Since the difference in control of the gene is chemical and does not involve sequences, it will not be detected by sequencing. In the words of Francis Collins, director of the US National Institutes of Health (NIH), "here is something where Mendel, Watson and Crick all seem to have missed some crucial goodies." Methylation can be imagined like a traffic signal, blocking the expression of some genes and enhancing the expression of others. At the moment we are studying these changes in DNA methylation and the genes that are altered in cancer only by DNA methylation. Some of this knowledge has reached clinical stages.

From single epigenetic biomarkers to epigenomics

As an example of therapy prediction in personalised cancer medicine, take the example of a particular marker, *MGMT*. Imagine that a patient goes to the doctor, who finds a tumour that is already metastatic. The doctor is unable to say what type of

tumour it is, where it came from, and therefore what the appropriate therapy is. But what if we could take a picture or obtain a fingerprint and compare it to a collection of pictures/fingerprints of known tumours? This would allow us to determine whether the patient's tumour resembles breast cancer, colon cancer, a brain tumour, etc. (Fig. 2) [8,9]

The tumour marker *MGMT* provides us exactly with this information, in the form of a DNA methylation profile of a tumour sample analyzed for this tumour marker—and this offers a strategy for many of the markers we know. Accordingly, further research, diagnostic development, and clinical trials will require synergies between academics, researchers, pharmaceutical and biotech companies, and between the public and private sectors.

MGMT is a gene that codes for a DNA repair enzyme, and we discovered that the respective gene is silenced—methylated—in cancer. We made this discovery in 1999, before the '-omics' revolution, by looking at candidate genes one by one and realizing that the *MGMT* gene is methylated in gliomas, which are tumours that start in the brain or spine. In its methylated state, MGMT cannot repair damaged DNA, such that tumours with methylated *MGMT* have more mutations and are more aggressive [3] We showed that gliomas with *MGMT* methylation are more sensitive to the family of drugs that includes procarbazine, dacarbazine, BCNU, and ACNU, because they target the same site in the DNA [4].

Ten years later, in 2009, in the '–omics' era, we were able to address this same issue using a micro-array, which identified *MGMT*, the same gene that we had found 10 years before. In parallel, scientists collaborating in the Cancer Genome Atlas Research Network arrived at the same conclusions [1]. This research was particularly satisfying because it yielded consistent results, with the effect of the *MGMT* gene validated using the new technology. Consequently, genomics seems to be a profitable route to finding new biomarkers for use in a personalised medicine approach to cancer.

Another example is *BRCA1*, a breast cancer susceptibility gene mapped to chromosome 17q21 by linkage studies. Defects in this gene are predicted to be responsible for 45 % of inherited breast cancers and >80 % of inherited breast and ovarian cancers. However, despite the high incidence of a loss of heterozygosity of the *BRCA1* region in sporadic breast and ovarian cancers, *BRCA1* somatic mutations have been found only in a few cases of sporadic ovarian and breast cancers. This suggests that an alternative mechanism for the inactivation of *BRCA1*, or other genes in its vicinity, plays an important role in the development of sporadic breast and ovarian cancers. Importantly, several labs found that a genetic alteration in *BRCA1* predicts that the tumour cells will be very sensitive to a family of drugs called PARP inhibitors [2].

While this is great news, the disadvantage is that out of a hundred patients with breast or ovarian tumours, only one or two will have this mutation, which in turn reveals one of the typical problems of personalised medicine: drug approval. The problem is actually two-fold, because if the drug is of interest for only a few patients, the economic benefits for the company that produces it will be too low to justify its production. How can we treat small populations of patients such that pharmaceutical companies can recover their costs?

In this particular case, we determined that it is not only the mutation but also the methylation state of the gene that predicts DNA damage and therefore the response to the drug. If 25 out of 100 breast cancer patients can be identified as responders, in this case to PARP inhibitors, the production of this drug may be justified, by introducing the right biomarker [5,6]. Thus, in clinical trials, the inclusion of biomarkers is critical because they will encourage the approval of effective drugs. Moreover, there are also many effective drugs from the past that can be rescued, if they are administered to patients tested
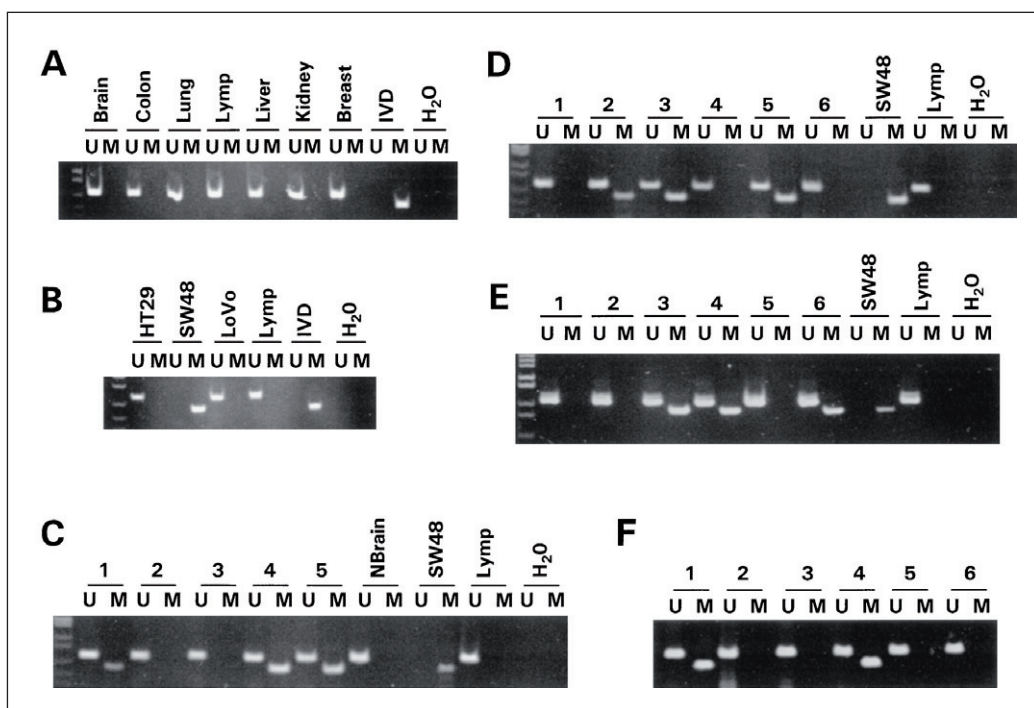

Fig. 2. Methylation Specific PCR (MSP) of *MGMT*. 'U' indicates the presence of unmethylated genes of *MGMT*; 'M' indicated the presence of methylated genes. (A) Normal Tissue. IVD: in vitro methylated DNA as positive control for methylation; H_2O: water control for PCR. (B) Cancer cell lines. (C) Primary gliomas. (D) Colorectal carcinoma. (E) Lung carcinoma. (F) Primary lymphomas. Source: [3]].

for the appropriate biomarkers.

Nowadays, we can use micro-arrays to obtain a complete DNA methylation fingerprint of any tumour. Over 20 methylated genes predict the responses to different chemotherapy drugs [12]. At the moment, however, only one such gene, *MGMT*, has been approved in Europe, to stratify patients with glioma. Other marker genes await approval for different reasons, e.g., they are very new, discovered in the last month, the last year, or the last 2 years. But in other cases there is insufficient interest on the part of pharmaceutical companies in their diagnostic development and other sources of investment are lacking. In the latter case, investment must come from the public arena, especially the European Union.

Thus, research leading to the discovery of new biomarkers for use in a personalised medicine approach to cancer must be supported. For example, the project called 'Curelung,' [www.curelung.eu] coordinated from our lab in Barcelona, has been funded in its totality by the FP7 program of the European Union. Its aim is to find biomarkers that predict the response to chemotherapy in lung cancer, which is still the most lethal type of cancer worldwide and specifically in Europe, where it accounts for 12.3 % of all new cancer cases every year. As noted in the first paragraphs of this article, lung cancer patients have a very poor prognosis, with a 5-year survival of only 14 %. One of the objectives of the project is to define the epigenetic markers that could determine the efficacy of or resistance to targeted therapies. Preliminary results indicate the stratification of lung tumours according to their genetic background, which translates into improved patient selection. We now have the technology not only to sequence complete genomes, but also to obtain complete epigenomes, complete methilomes. In 5 years we will probably be able to understand much of the data generated in these types of analyses.

Final considerations on personalised cancer medicine

To end this discussion on personalised medicine in the treatment of cancer, I would like to offer five considerations. First, is the need to keep in mind that tumours are heterogeneous. This means that even within a tumour there are small populations of cells that differ from the majority. Thus, even if we have a good biomarker for, say, 90 % of the cells, the remaining 10 % will be different and are likely to be the source of a relapse, with reappearance of the tumour. The answer is likely to be personalised cancer therapy that differs according to disease stage: primary tumour, metastasis, relapse, etc. Second, it is important that good drugs, both new and recycled, receive timely approval and that all clinical trials include the use of a biomarker, if at all possible. Very good drugs are already available. But if they are given to all patients at the same disease stage, their true benefit may not be appreciated. However, if biomarkers predict the efficacy of the drug for the cancer stage of a particular patient, even if the drug is an older one he or she should receive it, even if it is no

longer under patent protection or its continued manufacture is not financially profitable for the drug company. Third, we must stop using inefficient drugs. For example, some recently approved drugs increase survival by only 3 weeks—can this truly be claimed as a statistically significant effect? As a scientist, I do not see the benefit from their continued use. And yet these drugs have been approved. Fourth, it is crucial to avoid toxicity to respect the quality of life of cancer patients. Some of the drugs used in cancer treatment are not only of extremely limited efficacy, they are highly toxic. Some patients prefer a shorter survival but one that offers a higher quality of life, allowing them to spend time with friends and family and to avoid unnecessary financial burdens. This decision must be honoured.

Personalised cancer medicine also translates into cost savings for the healthcare industry as it can avoid useless treatment while freeing up resources for those patients likely to respond. Pathologists, for example, often ask me, "How can I convince my hospital director to implement personalised cancer medicine in my hospital?" The use of IT and cost-benefit analyses are powerful tools for convincing hospitals that personalised medicine is not the way of the future; it should be implemented now.

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