PROJECT DELIVERABLE

Validating Predictive Models and Biomarkers of Radiotherapy Toxicity to Reduce Side-Effects and Improve Quality-of-Life in Cancer Survivors

Project acronym: **REQUITE**	GA number: **601826**
Project title: **Validating predictive models and biomarkers of radiotherapy toxicity to reduce side-effects and improve quality-of-life in cancer survivors**	
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Project start date: **01 October 2013**	Duration: **60 months**
Project's coordinator: **Prof Catharine West (University of Manchester, UK)**	

Deliverable no.: **D4.2**	Title: **Report of patient recruitment on REQUITE website**
Due date: **Month 36 (30 September 2016)**	Actual date: **Month 41 (27 February 2017)**
Aim of the Deliverable: Produce report for numbers of apoptosis assays completed and provide initial descriptive analysis on REQUITE website (Task 4.1) **Deliverable D4.2 has been achieved in full**.	
Lead beneficiary for this deliverable: **Chris Talbot (B4)**	
Personnel involved: **Chris Talbot (B4); David Azria (B7); Carsten Herskind (B14).**	

Dissemination level:		
PU	Public	**X**
PP	Restricted to other programme participants (within the Commission)	
RE	Restricted to a group defined by the consortium (including the Commission)	
CO	Confidential, only for members of the consortium (including the Commission)	

The RILA test for prediction of adverse reactions to radiotherapy in the REQUITE project

Summary

There is previous evidence that a blood test called the RILA test can be used to predict side effects from radiotherapy. The REQUITE project is trying to confirm this in large numbers of breast, prostate and lung cancer patients.

Early experiments helped to standardise the test across the three European universities where the test was being carried out: Leicester, Montpellier and Mannheim. Further testing was then carried out in over 1300 patients, with similar results being found in the three laboratories. It was discovered that various factors affected results of the test, the most important being cancer type and whether the patients smoked. This will be important for future use of the test.

As the REQUITE project continues we will be able to determine whether the test does predict radiotherapy side effects. That would allow future clinical trials that optimise a patient's treatment based on their personal risk of serious side effects.

Introduction

The RILA (radiation-induced lymphocyte apoptosis) test has been demonstrated to have utility in predicting adverse reactions to radiotherapy. Studies by David Azria and colleagues showed that there was substantial variation between patients in the amount of cell death (apoptosis) in their blood cells (lymphocytes) exposed to radiation. The patients with the lowest amount of cell death (low RILA score) had worse adverse reactions than those with the highest amount of cell death (high RILA score). That is there is an inverse correlation between RILA score and side effects of the treatment. Several other research groups have confirmed this finding but it needs further verification in carefully controlled experiments before it ready for widespread clinical use.

The RILA test needs a small sample of fresh blood from the patients, which is then mixed with cell culture fluid and kept in a 37°C incubator for about 24 hours. Different flasks of the diluted blood is then either exposed to a high dose of X-ray radiation or left unexposed. Following the irradiation the samples are returned to the incubator for a further 48 hour period to give time for the blood cells to react to the radiation. At the end of this period the blood cells are separated from the fluid and stained with an antibody to identify the lymphocytes and a dye which quantifies the amount of DNA in the cell. Apoptotic cells (those that are in the process of being killed by the radiation) have less DNA and we can therefore count the proportion of lymphocytes that are affected. The RILA score is the difference between the amount of cell death in the irradiated and unexposed samples for each patient.

In the REQUITE project three of the clinical recruitment centres are carrying out the RILA test on blood samples from the breast, prostate and lung cancer patients: Leicester (UK), Mannheim (Germany) and Montpellier (France). This is part of Work Package 4 of the REQUITE project, task 4.1.

From the start of the REQUITE project in October 2013 there was a six month period in which the test was set up in each of the three centres, the laboratory protocol was standardised and checks were carried out to compare the results between centres. The first patients were recruited in May 2014 and the test was then performed throughout the recruitment period. The end date for carrying out the tests was extended in line with the extension of patient recruitment to the end of September 2016.

Protocol optimisation

At the beginning of the project the RILA test was already being performed in Montpellier, and with a different protocol in Mannheim, but not in Leicester. All three centres adopted the Montpellier protocol which was adapted into a standard operating procedure (SOP).

Over the first six months a series of trials were carried out to investigate:

1. Whether the timings of the blood incubations are important for RILA results
2. The degree of standardisation needed of laboratory reagents and equipment
3. How similar the results were between the three centres

These experiments determined that the length of the incubations before and after irradiation of the samples have an important effect on the results. The timings need to be carefully controlled and recorded – for this purpose a data collection form designated RQ9 was created.

It was found that the source of one of the tissue culture fluids called FBS had an effect on results and so one manufacturer's supply was specified in the SOP. Some compromises were necessary e.g. the three centres used different machines to measure cell death and this could not be standardised given the cost of purchasing new machines.

Two inter-laboratory comparisons were carried out, the first involved dispatching two blood samples from Leicester to the other centres, and the second involved sending six samples. These comparisons showed that the changes to the SOP helped reduce differences between the centres but that these differences were not removed entirely.

Testing of patient samples

By the end of September 2016 the three centres had carried out the RILA test in duplicate on a total of 1319 samples (see Table 1). These were 85% of all the patients recruited by the three centres: 67% at Leicester, 71% at Mannheim and 96% at Montpellier.

Table 1: Number of RILA tests carried out by centre and cancer type

Centre	Breast	Lung	Prostate	Total
Leicester	204	25	199	428
Mannheim	147	0	61	208
Montpellier	411	27	245	683
Total	**762**	**52**	**505**	**1319**

The RILA test results are used to calculate a RILA score, which represents the increase in cell death as a result of radiation. The results of the RILA score for each centre were broadly similar in terms of average, range, risk cut-off points and distribution (Table 2 and Figure 1):

- Leicester 21.4% (Range 2.2-70.8%). Cut-off points 14.5% & 24.5%.
- Mannheim 21.5% (Range 2.2-69.2%). Cut-off points 15.3% & 25.1%.
- Montpellier 17.9% (Range 2.1-68.8%). Cut-off points 11.9% & 20.6%.

Tertile cut-off points could be used to divide the patients into three equal groups of low, mid and high radiation-induced cell death.

There was evidence for differences between cancer types, a novel finding not previously reported, with lung cancer patients having lower RILA scores than breast or prostate patients (Table 2). This observation may be important when applying the test for use in clinical trials, with different cut-offs being used for each cancer type.

Table 2: Average RILA scores by centre and cancer type. Mannheim does not recruit lung cancer patients

Centre	Breast	Lung	Prostate
Leicester	19.9%	13.9%	25.6%
Mannheim	22.2%	N/A	20.1%
Montpellier	19.7%	11.2%	15.3%

No significant difference was found between centres for breast or lung cancer patients, but the inter-centre difference is significant for prostate cancer patients. The explanation for this is probably differences in the pre-radiotherapy treatments the patients receive. For example a higher proportion of the Leicester prostate cancer patients have received hormonal therapy than in Montpellier.

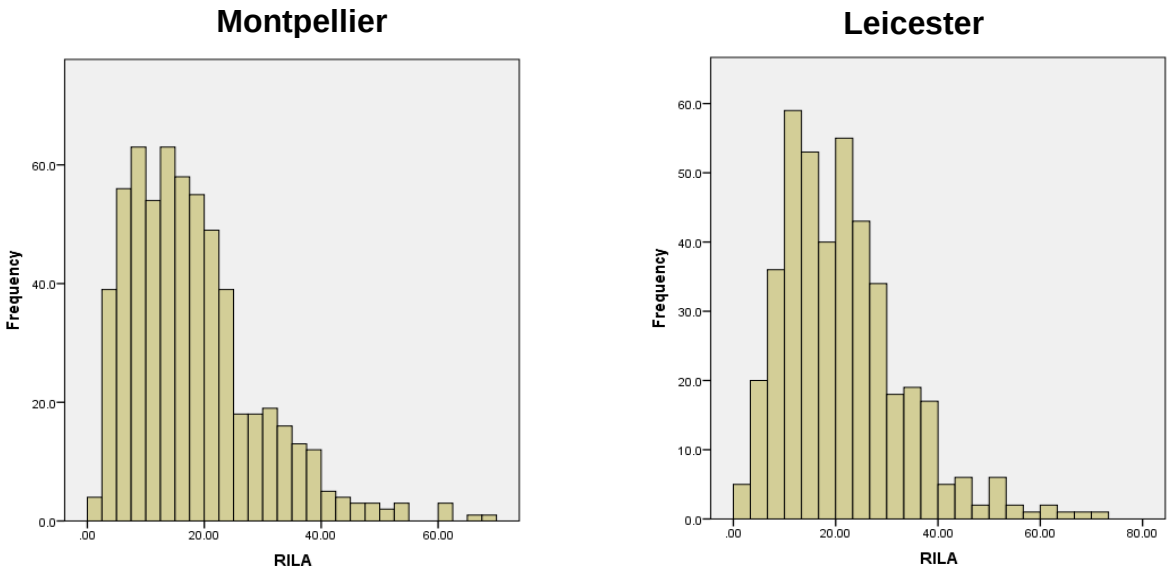

Figure 1: Distribution of RILA results for the two larger centres, combining all three cancer types. The shape of the curves looks similar, suggesting that the test was successfully standardised.

An analysis of patient-related factors that affect RILA testing shows that multiple variables have an independent effect:

- Patients with higher proportion of one type of lymphocytes have increased RILA scores. This may be important to the mechanism underlying the predictive value of the test.
- Smoking: Never & ex-smokers have an average RILA score 23.1%, compared with recent & current smokers who have an average of 18.0%. This may well be a con-founding factor that will need to be corrected for when the RILA test is used as a predictive test.
- Depending upon the cohort, other treatments the patients have previously or are currently taking appear to affect RILA, including anti-depressants, alpha blockers & prior hip replacement.

Future analysis

Data on the irreversible side effects of radiotherapy treatment will not be available until two years after the patients were treated, delaying the final analysis into the latter part of 2018. An interim analysis will be carried out in 2017 to study whether RILA score is associated with acute reactions to radiotherapy e.g. skin peeling in breast cancer. Previous evidence has not suggested that RILA predicts acute reactions, but this large dataset may be able to detect some effect.

If the REQUITE project validates that the RILA test can predict radiotherapy reactions then it can form the basis of future clinical trials in which patients will be given the optimal treatment depending upon their personal risk of suffering bad reactions.