PREDICTING BENEFIT FROM INTERFERON TREATMENT: PERSONALISED THERAPY FOR MELANOMA

A collaborative study between the University of Leeds, the AIM-HIGH Study Group, the EORTC Melanoma Group, Nordic Interferon Trial and the FP6 funded group, Chemores.

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Version 4 – 02/09/08

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2. Synopsis

Study Title
Predicting Benefit from Interferon Treatment: Personalised Therapy for Melanoma

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Study Centres
- The University of Leeds
- The EORTC Data Centre
- Erasmus University
- The Karolinska Institute

Estimated Study Period
2008-2012

Background and study rationale
The probability of survival from cancer and response to treatment are likely to be affected by hereditary variation in the genome and genetic changes in the tumour.

Stage IIB-III melanoma 5 year survival rates are only 40-50% (Gimotty *et al.* 2005). Melanoma cells induce immuno-suppression and defects in interferon signaling are an important component of this suppression (Wheatley *et al.* 2003). Interferon therapy is the most widely used adjuvant therapy worldwide for patients with disease at a high risk of recurrence after resection. Meta-analysis of randomized controlled trials demonstrates that interferon prolongs relapse free survival time in these patients (Wheatley *et al.* 2003), however the side effects of interferon limit its use and currently we cannot predict who will obtain an overall benefit from the treatment. Development of autoimmune features during interferon treatment appears to be associated with improved response (Gogas *et al.* 2006; Satzger *et al.* 2007) and toxicity associated with interferon appears to be due to release of cytokines (Kirkwood *et al.* 2002). Predisposition to development of autoimmune features and magnitude of cytokine production with interferon treatment could be influenced by genetic changes in both germline DNA and tumour DNA. Therefore we aim to identify genetic biomarkers that predict whether a patient will obtain some benefit from interferon treatment and biomarkers that will predict development of toxicity. This will potentially help identify those patients who will gain an overall benefit from the treatment and help avoid toxicity in those who will not respond. This will be a step towards personalizing therapy for patients with melanoma. As melanoma is an immunogenic tumour, exploring genetic changes in both the primary tumour and the germline will increase our understanding of interaction between host and tumour.

A number of genetic changes have already been identified in the hereditary genome which appear to influence the survival of the patient irrespective of the treatment they receive. Therefore, a further aim of this study is to address this in more detail with the aim of identifying genetic biomarkers which predict survival in melanoma which could potentially be used prognostically in the future.

Study Objectives
To undertake a large collaborative study using primary tumour, lymph node samples or DNA extracted from blood from patients with stage IIB to stage III melanoma with the aims of:

(1) Identifying biomarkers that predict response from interferon therapy
(2) Identifying biomarkers that predict development of toxicity
(3) Identifying genetic biomarkers that predict survival from melanoma irrespective of therapy
(4) Correlating lymphocyte gene expression profiles with germline variation in immune response genes, to understand genetic control of immunological responses to cancer.

Findings from this study will contribute to the development of personalized therapy for patients with melanoma.

Study Design

Survival analysis of patients with stage IIB to III disease by germline genotype and gene expression in tumour cells and normal lymphocytes. The participants have been previously recruited to trials of adjuvant interferon therapy in which they were either randomized to receive interferon or have no adjuvant treatment after surgical resection.
Samples from patients recruited in the UK will be collated and analyzed in Leeds. European samples are being collected and analysed in Europe. Data sets will be analysed separately and any significant findings will be validated in the other data set. Results will then be pooled and significant results validated in a further data set (from Dr. John Kirkwood's group). This protocol describes the study design in the UK.

Study Participants

Numbers of participants

Patients involved in the following trials will be recruited to this study:

- AIM HIGH (Hancock *et al.* 2004)
- EORTC 18952 (Eggermont *et al* 2005)
- EORTC 18991 (Eggermont *et al.* 2008)
- The Nordic Interferon Trial (Hansson *et al.* 2007) (In Europe only)

Overall, 4173 patients were recruited to these trials as shown below. 1143 patients were recruited in the UK and 3030 in Europe. Power calculations are based on the assumption that we will be able to retrieve 90% of tumour blocks from UK patients and 25% from other European patients, based on our previous success rates in the UK and conservative estimates for European samples. This will give 1028 UK samples and 757 other European samples from the trials.

Inclusion criteria

All patients recruited to the above trials will be included.

Exclusion criteria

None.

Study design

Identification of potential participants

In the UK, for patients involved in the 'Aim High' and EORTC trials, patients' details will be provided from the clinical trials office to the clinicians who randomized the patients to the trial.

Tracing samples

The relevant clinicians in each UK centre will then trace the patients' primary tumour with wide local excision and lymph node blocks if available. Extracted DNA from blood samples for use in ethically approved research will also be traced. The samples will be re-labeled with study number and date of birth only in the recruiting centre.

Obtaining clinical data

Data regarding the patients' original melanoma and nodal involvement, their clinical course following randomization and whether they were randomized to interferon or observation will

be obtained from the 'Aim High' clinical trial office and the EORTC data centre and transferred to Leeds labeled by study number and date of birth only.

Tissue sampling and analysis
Cores of tumour will be taken from either the primary or metastatic deposits in lymph nodes (or both). RNA will be extracted for use in expression arrays and DNA will be extracted for mutation screening. Cores of normal epidermis or lymph nodes without metastatic deposits will be taken for extraction of germline DNA, to assess hereditary genetic variation using single nucleotide polymorphisms. If available, germline DNA from blood could also be used. RNA will be extracted from normal lymphocytes in nodal samples. Slides and cores will be taken for future immunohistochemistry to validate any findings.

Data analysis
Variation in germline DNA will be analysed as determinants of OS, RFS and development of interferon toxicity. Variation in germline DNA, tumour DNA and tumour and lymphocyte RNA expression will be analysed in terms of effect on RFS/OS.

Primary outcome measure
Relapse free survival time (RFS)

Secondary Outcome measure
Overall survival time (OS)

Statistical methods
Power calculations are based on the assumption that we will be able to retrieve just over 1000 UK samples and 750 other European samples from the four trials, and we have assumed a 55% relapse rate. Using all samples there will be 73% power at a 1% significance level and 89% power at a 5% significance level to detect an interaction between treatment with IFN and a biomarker with 50% prevalence, assuming a 50% increase in median relapse-free survival time in patients with the biomarker. Any candidate marker identified in this analysis will require validation in a further data set.
Candidate biomarkers of response to IFN therapy will be investigated in UK and European samples separately, with significant findings being validated in the other group. Both data sets will then be combined for analysis.

Expression array data
Microarray data will be screened using the SAMROC method (Borberg 2003) to identify differentially expressed genes in different groups of patients (relapser/non-relapser, responders to IFN/non-responders etc.). This method uses a penalized t-test that is robust to outliers, minimizes false positive and false negative rates and has good power (Broberg 2003; Kim *et al.* 2006). The concordance between the expression levels from the DASL 3 probes per gene per sample will be assessed and, where appropriate, their average will be used (Fan *et al.* 2004).

Analysis of prognostic biomarkers
For each SNP, evidence for a relationship with RFS or OS will be examined using log rank tests. For biomarkers showing some evidence of a relationship with outcome (SNPs or differentially expressed genes), hazard ratios (HRs) and 95% confidence intervals (CI) will be estimated using single variable and multivariate Cox proportional hazards models, adjusting for other factors previously shown to be of prognostic importance in melanoma (ulceration of the primary tumour, depth of primary tumour (Breslow thickness), number of nodes involved and sex) and for treatment group; the analyses will be stratified by study as in a meta-analysis. Polymorphisms in linkage disequilibrium within each candidate gene, where there is some evidence of an effect on outcome, will be combined in haplotype analyses.

Analysis of whether biomarkers are predictive of treatment effect and toxicity
For each candidate predictive biomarker, Cox proportional hazards models stratified by study

will also be used to estimate HRs and 95% CIs for the effect of treatment group (interferon (IFN) versus observation), separately by each category of the marker, to see whether the HR varies by biomarker status. Interactions between treatment effects and biomarkers will be formally tested using the likelihood ratio test (comparing a survival model including a treatment/marker interaction term and main effects with a model including only main effects). A similar approach will be taken to analyse candidate biomarkers that predict toxicity (defined as myelotoxicity, transaminitis, depression and constitutional). Each factor will be examined individually as will a combined measure of these factors.

Multiple testing
Because of the multiple testing to be carried out in this study, any positive findings will require independent validation. Findings significant at the nominal 5% significance level will be investigated further. As a guide to interpretation of results in the context of multiple testing, false positive report probabilities (FPRP) will be calculated according to Wacholder (2004). The FPRP estimates the risk that a statistically significant interaction is false-positive, based on the observed p-value, the power to detect interaction at that level and the prior probability (PP) of interaction. PP is a subjective measure, used to reflect the strength of the prior hypothesis and preceding data, but applying a range of PPs can test the robustness of any statistically positive findings.

Development of a classification algorithim
In a second approach we will take a more speculative route and attempt to develop a classification rule to identify patients likely to (1) benefit from IFN therapy in terms of survival, (2) develop less toxicity. In this analysis, UK patients will be used to develop the classification rule. Gene expression data and large scale SNP data will be analysed using Random Forests, a classification method that often out-performs most alternatives and avoids overfitting by providing unbiased estimates of predictive performance based on “out-of-bag” samples. If a rule can be developed with good predictive properties, this will be further validated in the non-UK samples. Further, we will examine the prediction of survival and toxicity but the precise approaches will depend upon the pattern of relationship.

3. Glossary of Abbreviations

Abbreviation used in text	Unabbreviated form
CI	Confidence intervals
DMFI	Distant metastasis free interval
DMFS	Distant metastasis free survival
DNA	Deoxyribonucleic acid
EORTC	European Organisation for Research and Treatment of Cancer
FPRP	False positive report probabilities
HR	Hazard ratio
IFN	Interferon
IL	Interleukin
MU	Million units
OS	Overall survival
PIAG	Patient Information Advisory Group
PP	Prior probability
R and D	Research and development
REC	Research Ethics Committee
RFS	Relapse free survival
RNA	Ribonucleic acid
SNPs	Single nucleotide polymorphisms
OS	Overall survival

4. Study Development Group

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5. Background

Survival from cancer and response to treatment is likely to be affected by hereditary variation in the genome as well as genetic changes in the tumour itself.

The incidence of melanoma continues to increase. With surgical treatment the prognosis in stage I disease is excellent. However, in those with disease classified at an intermediate or high risk of recurrence (stages IIB, IIC and III) survival rates at 5 years following surgery are only 40-50% (Molife *et al.* 2002; Gimotty *et al*. 2005). Surgical resection is the mainstay of treatment, however in those with high-risk disease an effective adjuvant could significantly reduce the number progressing to stage IV disease. Despite extensive studies over the last 30 years, only IFN treatment seems to improve relapse free survival time, but the toxicity associated with this limits it use and so currently there is no standard systemic adjuvant therapy recommended in Europe for those with resected high risk melanoma (Molife *et al.* 2002; Roberts *et al.* 2002). This project will allow us to identify biomarkers of benefit and therefore give patients with melanoma access to this therapy in the future.

5.1. Adjuvant IFN therapy

IFN-! therapy is currently the most widely used adjuvant therapy worldwide in the treatment of melanoma. Melanoma cells induce immuno-suppression and defects in IFN signaling are an important component of immune suppression (Wheatley *et al.* 2003). The type 1 interferons of which IFN-alpha is one, have pleiotropic effects and the evidence suggests that the dominant anti-tumour effect is immunomodulatory although IFNs are anti-angiogenic and have direct antiproliferative effects on the tumour. (Moschos and Kirkwood 2007).

5.1.1. Benefits and risks of IFN Therapy

A number of randomized trials have demonstrated a benefit of using adjuvant IFN following resection of high risk disease, most notably IFN therapy appears to extend disease free survival time (Pehamberger *et al.* 1998; Grob *et al.* 1998). Conversely, other large randomized trials have demonstrated no benefit of adjuvant IFN treatment (Cascinelli *et al.* 2001; Cameron *et al.* 2001). When data from these trials are analysed together in a meta-analysis it is clear than adjuvant IFN therapy improves RFS, but has a less clear impact on OS (Wheatley *et al.* 2003). Therefore, IFN therapy appears to extend RFS time in high-risk disease, but this benefit is

limited by the toxicity of the drug.

IFN therapy is associated with significant side effects including flu-like symptoms (80%), myalgia and arthralgia (75%), anorexia (70%), depression (40%) and hepatic effects (35%) (Eggermont *et al.* 2005; Gogas *et al*. 2006; Hancock *et al.* 2004). This toxicity can lead to up to 20% of patients being unable to tolerate the therapy (Chiarion-Sileni *et al.* 2006; Eggermont *et al.* 2005; Hancock *et al.* 2004), and in those that continue, IFN treatment can have a detrimental effect on the quality of life of patients (Dixon *et al.* 2006; Rataj *et al.* 2005).

Therefore, the overall benefit is unclear as the effect on RFS is relatively small and toxicity for many is considerable. Being able to identify the patients who are most likely to respond to this treatment will help clinicians choose patients who will obtain an overall benefit from IFN therapy and avoid toxicity in those unlikely to respond.

5.1.2. Predictors of response to IFN Therapy

Previous studies have demonstrated that development of autoantibodies or clinical autoimmune disease is associated with a better response to IFN therapy (Gogas *et al.* 2006; Satzger *et al.* 2007). Controversy exists as to the significance of this finding (Bouwhuis 2007a and 2007b), but as the tendency to auto-immunity is at least in part genetically controlled, the finding is consistent with the view that in those who develop autoimmune features and respond well to IFN there is hereditary variation in their genome which moderates host tumour interactions.

Immunomodulatory effects of IFN-alpha may be mediated by Toll Like Receptors (TLR), nuclear factor kappa-B (NF- B), activating protein 1 (AP-1) and interferon regulatory factor 7 (IRF-7) at a cellular level by plasmacytoid dendritic cells and lymphocytes. IFN's regulate a number of different signaling pathways classically the STAT family of proteins which moderate transcription of a number of genes (Moschos and Kirkwood 2007). In melanoma and other cancers, variation in levels of related proteins such as suppressors of cytokine signaling (SOCS) have been reported to correlate with tumour responsiveness (Roman-Gomez *et al.* 2004; Sakai *et al.* 2002; Zimmerer *et al.* 2007). The myxovirus-resistance protein (MxA) is produced only in response to IFN in pharmacologic responders and was recently suggested as a biomarker of response in patients with multiple sclerosis to IFN (Malucchi *et al*. 2008). Therefore there are a number of candidate genes in these

pathways which may explain hereditary variation in benefit from IFN treatment.

At the somatic level, the effects of IFN are mediated by the IFN! # receptor so that variation in the germline gene coding for the receptor or loss of expression in the tumour will moderate response. The antiproliferative effects of the type 1 IFNs may be mediated by a number of pro-apoptotic ligands such as Fas ligand, TNF-related apoptosis inducing ligand (TRAIL) and death inducing signaling complex (DISC) (Pokrovskaja *et al.* 2005). There are some data from small studies that expression of methylthioadenosine phosphorylase (*MTAP*) gene in a tumour may act as a potential predictive marker of response to IFN, however this requires confirmation in larger studies (Behrmann *et al.* 2003; Wild *et al.* 2006, 2007).

5.1.3. Predictors of toxicity with IFN therapy

As discussed previously, IFN is associated with significant side effects. IFN causes release of a cascade of cytokines including tumour necrosis factor-! (TNF-!), IL-1, IL-2, IL-6, IFN- and IFN inducible protein 10. These cytokines initiate cellular processes that produce many of the observed toxicities associated with interferon therapy including constitutional symptoms, fatigue, mood disorders, cognitive changes and anorexia (Kirkwood *et al.* 2002). Interferon can also cause significant hepatotoxicity, the mechanism of which is poorly understood. It has been demonstrated that IFN-! suppresses activity of specific cytochrome P450 (CYP) isoenzymes (Knickle *et al.* 1992). There is a significant association between the magnitude of the inhibition of CYP isoenzymes by IFN and the development of toxicity (Islam *et al.* 2002).

Both the level of cytokines produced with IFN treatment and the sensitivity of CYP isoenzymes to IFN could be genetically determined and variable between individuals. Identification of these variations may predict those patients more susceptible to toxicity.

5.2. Survival in melanoma

A number of mutations and polymorphisms have been identified in the genome, which appear to influence the prognosis of patients with melanoma independent of the treatment that the patients have received. These genetic alterations include vitamin D receptor polymorphisms, and polymorphisms in interleukin genes (Hutchinson *et al.* 2000; Kumar *et al.* 2003). These findings support the view that

hereditary genetic variation contributes to the prognosis for patients with malignant melanoma.

In this study, a further aim is to identify hereditary variation predictive of survival irrespective of treatment. Being able to identify markers of survival from melanoma independent of treatment will help provide prognostic information to patients and allow stratification of patients into groups at high risk or low risk of relapse which will allow modification of their treatment accordingly. Furthermore the identification of genes which impact on survival will give insight into host tumour interactions which may inform therapeutic options in the future.

5.3. Rationale for the study

Survival in patients with disease at high risk of relapse (stage IIB to III) could be significantly improved by an effective adjuvant for use after resection of the primary tumour and involved lymph nodes. IFN therapy is currently the only adjuvant which appears to improve relapse free survival time, but toxicity limits it use. This study aims to identify genetic biomarkers that will predict benefit from IFN therapy and development of toxicity. The size of this study will ensure we have enough power to detect genetic variations which may influence response to IFN therapy. These findings could potentially be used to identify patients who will obtain an overall benefit from the therapy, while avoiding unnecessary toxicity in those who will not respond. Melanoma is a immunogenic tumour, and exploring the genetic changes in both primary tumour and germline will increase understanding of interaction between host and tumour.

As we have discussed, the incidence of melanoma is increasing and there is some evidence that a number of germline hereditary variations appear to influence survival in melanoma irrespective of the treatment a patient has received. To date, studies that have identified these variations have been small and underpowered. This study aims to address this by undertaking a large collaborative study with sufficient power to detect variations which influence relapse free survival time. Potentially these genetic variations could be used to stratify patients appropriately into high risk and low risk groups and ensure they receive appropriate treatment.

6. Hypotheses

The hypotheses we wish to address in this study are that:

(1) Hereditary variation along with somatic events in the tumour influences the response of the tumour to IFN therapy,
(2) Hereditary variation influences development of toxicity from IFN therapy, and
(3) Hereditary variation moderates survival from melanoma irrespective of treatment.

7. Aims

We plan to perform a large scale collaborative study using samples from patients with stage IIB to III melanoma who have been previously involved in large randomized trials which aimed to identify improvement in RFS and OS with IFN therapy.

The aims are to:

(1) Identify genetic biomarkers that predict benefit from IFN therapy,
(2) Identify biomarkers that predict development of toxicity,
(3) Identify biomarkers that predict survival from melanoma irrespective of therapy, and
(4) Correlate lymphocyte gene expression profiles with germline variation in immune response genes, to understand genetic control of immunological responses in cancer.

We anticipate that the findings from this study will inform the first step towards personalized therapy for melanoma patients.

8. Study Design

Survival analysis of melanoma patients with stage IIB to III disease by germline genotype and gene expression in nodal metastases, primary tumour or lymphocytes, in patients treated in adjuvant therapy trials which have reached maturity: the 'Aim High' trial (Hancock *et al.* 2004), the EORTC pegylated interferon trial 18991 (Eggermont *et al.* 2008), the EORTC 18952 trial (Eggermont *et al.* 2005) and the

Nordic Interferon study (Hansson *et al.* 2007).

This is a collaborative study with our European colleagues. In Leeds our role will be to identify those recruited in the UK to the interferon trials and analyze their samples. Within Europe, Chemores in collaboration with the EORTC will collect European samples relevant to this study and samples will be analysed in Rotterdam under the supervision of Professor Eggermont. Data from the two groups will initially be analyzed separately with any strongly predictive biomarkers being identified in either group being validated in the other set. The data from the two groups will then be pooled for analysis to increase the power of the study to detect clinically relevant biomarkers. Any significant biomarkers identified in this process will be validated using another data set in collaboration with Dr. John Kirkwood of ECOG and the University of Pittsburgh, USA, who has carried out the majority of the USA trials on adjuvant IFN therapy in melanoma.

Within the Leeds group, Professor Eggermont's group, the EORTC and Chemores, anonymised samples, DNA and RNA, tissue microarrays and clinical data will be exchanged.

The following research plan describes the process for patients recruited to trials in the UK.

9. RESEARCH PLAN

9.1. Potential participants and samples

9.1.1. Samples

We will access paraffin embedded stored primary tumours, wide local excision samples, lymph nodes from block dissection or extracted DNA from blood samples from patients previously recruited to randomized controlled trials in which patients were randomized to IFN treatment or observation as detailed below. From these samples we will extract DNA and RNA. We will also take sections or cores of the tissue for immunohistochemistry or development of multitumour arrays to validate findings in the expression array studies, and for independent pathological review.

9.1.2. Clinical trials

In the UK we plan to obtain samples from patients recruited to the 'Aim High' and EORTC trials:

- The 'Aim High' study (Hancock *et al.* 2004)

This was a study of low dose extended duration IFN as adjuvant therapy in patients with completely resected high-risk melanoma, the objective being to determine the effects of interferon alfa-2a on OS and RFS. Other secondary objectives included analysis of interaction between effect of interferon therapy, age and sex on RFS and OS and to document side effects of IFN therapy according to National Cancer Institute Common Toxicity Criteria (Oken *et al.* 1982).

Six hundred and seventy four patients with completely resected melanoma at a high risk of recurrence were recruited between 3rd October 1995 and 22nd December 2000. To be eligible for the study, patients were to: (1) be fit enough to receive IFN if allocated, (2) have had a resection less than 12 weeks prior, (3) have a healed surgical wound, (4) have no other history of malignant disease (5) not be pregnant, lactating, or intending pregnancy during treatment (6) have had no previous biologic therapy (7) not be taking systemic corticosteroids or other immunosuppressive therapy, and (8) to have given written informed consent.

The target accrual of patients was 1000 patients through a period of 5 years, which would give a 90% chance of detecting (at a 2-tail P value of 0.05) a 10% absolute difference in RFS and OS. However, on the advice of the data monitoring committee,

the study was terminated after 5 years with 674 patients recruited, as it was felt that extending the period of recruitment to increase the number of patients involved would not statistically change the analysis. Of the 674 patients recruited, 338 patients were randomized to receive IFN and 336 were randomized to observation alone (no adjuvant treatment). Random assignments were balanced by minimization using the following subgroups: age group (<50 or ≥50 years), sex, disease status (primary tumour ≥4mm, resected nodes at initial presentation, resected nodes at subsequent relapse, non-nodal regional recurrence) and treatment centre.

The group randomized to receive IFN was given 3 MU of IFN alfa-2a three times a week until recurrence or until 2 years (whichever occurred first).
Median duration of follow up until 31st July 2002 and time of initial analysis was 3.1 years (range: 0-6.8 years), however follow-up continues and some patients have now 12.3 years of follow-up.

Of the patients recruited 130 had localized disease, 74 had locally metastatic disease, 85 had regionally metastatic disease at diagnosis and 385 has regionally metastatic disease at recurrence. The study demonstrated no significant difference in OS or RFS in the IFN treated or control arms (OS: odd ratio 0.94, 95% CI 0.75-1.18, p=0.6 and RFS: odds ratio 0.91, 95% CI 0.75-1.10, p=0.3). Moderate IFN toxicities were noted (7% in IFN group, 4% in observation group), the majority being grade 3 fatigue or mood disturbances. However, 15% of patients withdrew due to toxicity.

- EORTC 18952 (Eggermont *et al.* 2005)

The aim of this study was to assess the effect of two regimens of intermediate dose IFN versus observation alone on DMFI and OS in patients with stage IIb or stage III disease. Toxicity associated with treatment was also recorded using NCI common toxicity criteria.

One thousand three hundred and eighty eight patients were recruited between the ages of 18-75 years. Patients either had stage IIB or stage III disease. Those with stage IIB disease had had their primary tumour (thickness ≥4mm) removed and those with stage III melanoma had had regional lymph node metastases resected. Patients excluded were those with mucosal or ocular melanoma, those previously treated with systemic drugs for melanoma, those with other malignant diseases (other than basal cell carcinoma, in situ cervical carcinoma), autoimmune disease,

uncontrolled infections, cardiopulmonary disease, liver and renal disease or those taking corticosteroids.

Patients were assigned to receive either 13 months (n=553) or 25 months (n=556) of treatment with subcutaneous IFN alfa 2b, or observation (n=279). Treatment was comprised of 4 weeks of 10 MU for 5 days per week followed by either 10 MU three times a week for 1 year or 5 MU three times a week for 2 years, to a total dose of 1760 MU.

The authors calculated they would require 1400 patents enrolled to detect a 10.5% difference in DMFI at 4 years at 0.05 significance level with 80% power between each treatment group and the observation group. Median follow-up was 4.65 years (range 1-7 years) at the time of definitive analysis. Of the patients recruited 356 had stage II disease with 353 having stage III disease with non-enlarged microscopically involved lymph nodes on sentinel lymph node biopsy and 679 patients with stage III disease with palpable tumour involved nodes. Of the patients with stage III disease 412 were randomized to receive 13 months of IFN therapy, 414 were randomized to 25 months of IFN therapy and 206 to observation only.

At 4.5 years, this study showed that there was no statistically significant extension in DMFI or OS time with either regimen of IFN treatment when compared to observation alone. The 25 month IFN group showed a 7.2% increase in rate of DMFI (HR 0.83, 97.5% CI 0.66-1.03, p=0.05) and a 5.4% improvement in OS (HR 0.85, CI 0.68-1.07, p=0.12). The 13 month interferon group showed a 3.2% increase in rate of DMFI at 4.5 years (HR 0.93, CI 0.75-1.16, p=0.48) and no extension of OS. High levels of toxicity were recorded in treatment groups (13 months: 85%, 25 months: 82%, observation: 15%), however the majority were grade 3 toxicities for constitutional symptoms and fatigue. Despite this, only 18% were taken off the study or refused treatment because of toxicity.

- EORTC 18991 (Eggermont *et al.* 2008)

The aim of this trial was to assess the efficacy and toxicity of long-term pegylated IFN alfa-2b versus observation only. The primary end points were RFS and DMFS, with secondary endpoints being OS and safety.

One thousand two hundred and fifty six patients were recruited all with stage III

disease, with either microscopic nodal disease (N1, n=543 patients) or with palpable nodes (N2, n=713 patients). Patients were then randomized to received pegylated IFN or observation only.

The randomization was stratified by microscopic vs. palpable nodes, 1 vs. 2-4 vs. 5 or more nodes, Breslow thickness, ulceration, gender and site of tumour.

The patients randomized to receive pegylated IFN, received induction over 8 weeks of 6μg/kg/week, then maintenance of 3μg/kg/week for 5 years or until distant metastases developed.

Patients were recruited between 2000 and 2003. The authors calculated that a sample size of at least 1200 patients would be needed to observe at least 576 distant metastases or deaths. With this number of events, the study would have approximately 90% power to detect a HR of 0.76 for DMFS, or a 9.75% difference (from 40% to 49.75%) at 4 years. Six hundred and twenty seven patients were randomized to receive pegylated IFN and 629 were randomized to observation only. The median follow-up of the patients was 3.8 years.

This study has demonstrated an improvement on RFS with pegylated IFN treatment (HR 0.82, 95% CI 0.71-0.96, p=0.01), but not on DMFS or OS. This effect on RFS appeared to be more pronounced in patients with microscopic nodal disease (N1) than those with palpable nodal disease (N2) (In N1 disease HR 0.73, p=0.016; in N2 disease HR 0.86, p=0.12). Grade 3 and some grade 4 toxicity (according to NCI common toxicity criteria) was reported in 45% of the treatment group and 12% of the observation group. 31% of patients stopped treatment as a consequence of toxicity.

In Europe, samples will be traced from patients recruited to the EORTC trials and the Nordic Interferon trial:

- The Nordic Interferon Trial (Hansson *et al.* 2007)

The trial was designed to investigate the efficacy and toxicity of adjuvant post-operative therapy with intermediate-dose IFN alfa-2b in patients with stage IIB-C/III cutaneous melanoma. The outcome of maintenance treatment with IFN for 1 versus 2 years following a 4 week induction period was studied. Between November 1996 and August 2004 a total of 855 patients were entered into the study. Patients were randomized in equal proportions to three study arms: Arm A: Observation only. Arm B: induction: IFN 10 MU S.C. 5 days/week for 4 weeks; maintenance: IFN 10 MU

S.C. 3 days/week for 12 months. Arm C: induction as arm B; maintenance: IFN 10 MU S.C. 3 days/week for 24 months.

Adjuvant IFN therapy significantly improved RFS. Median RFS for Arm A: 23 months: Arm B: 38 months; Arm C: 29 months. HR for recurrence for IFN treated patients (Arms B + C combined) was 0.83 (p=0.049) compared to Arm A. There was no significant difference in RFS between arms B and C (1 and 2 years of maintenance therapy, respectively). Adjuvant IFN therapy had no significant effect on OS. HR for death for IFN treated patients (Arms B + C combined) was 0.88 (p=0.22) compared to Arm A.

9.1.3. Inclusion criteria

Patients recruited to any trial will be eligible.

Within the UK, centres which recruited patients to these studies and have agreed to collaborate are: the Christie, Birmingham, Sheffield, Leeds, Salisbury, Oxford, Glasgow, St. Georges, Newcastle, Dundee, Cambridge and Cheltenham.

We are in discussion with other centres in the UK who enrolled patients into the interferon trials including the Royal Marsden, Southampton and Bristol.

9.1.4. Exclusion criteria

There are no exclusion criteria.

9.1.5. Number of samples/patients

Overall, 4173 patients were recruited to these trials as shown below. 1143 patients were recruited in the UK and 3030 in Europe.

Based on previous retrieval rates by our group, we estimate that we will retrieve 90% of the blocks from patients recruited in the UK, which gives us a total of 1028 samples. From Europe we expect our retrieval rate to be lower at 25%, which gives an additional 757 samples. Therefore in total 1785 patients will be included in this study.

The total number of patients recruited to the IFN trials with stage II disease was 1049 and we aim to retrieve 418 of these samples. A total of 3124 patients with stage III disease were recruited, we aim to obtain 1367 samples.
Power calculations are detailed in section 10.

UK Patients

Trial	Total UK recruited patients	Stage II disease (90% retrieval)	Stage III disease (90% retrieval)	Cumulative UK total (90% retrieval)
AIM HIGH	674	204 (184)	470 (423)	607
EORTC 18952	142	36 (32)	106 (95)	734
EORTC 18991	327	0	327 (294)	1028
Total	1143	240 (216)	903 (812)	

With European Patients

Trial	Total recruited patients in Europe	Stage II disease (25% retrieval)	Stage III disease (25% retrieval)	Cumulative total with UK patients (25% retrieval)
AIM HIGH	0	0	0	1028
EORTC 18952	1246	325 (81)	921 (230)	1339
EORTC 18991	929	0	929 (232)	1571
Nordic	855	484 (121)	371 (93)	1785
Total	3030	809 (202)	2221 (555)	

9.2. Identification of potential participants

As previously stated, potential patients and samples will be identified from the above trials designed to assess the benefit of adjuvant interferon therapy in malignant melanoma. Identification will occur at each trial centre.

From the 'AIM HIGH' trial:
Patient details (name, dates of birth, hospital of recruitment, hospital number, date of randomization, study number) will be provided in a data file to the clinicians who randomized the patients by the clinical trials office in Sheffield for the 'Aim High' trial.

For the EORTC 18991 and 18952 trials:
Patient details (initials, date of birth, hospital of recruitment, hospital number, date of randomization, study number) of those recruited to EORTC 18991 and 18952 trials will be sent to the UK clinicians who randomized the patients to the trial.

This data will be sent by encrypted e-mail or mailed securely to the recruiting centres.

9.3. Tracing samples

For eligible participants, the relevant clinicians in each centre will then trace the blocks of primary tumour and any wide local excision and lymph nodes samples. A few centres have extracted DNA available from blood samples taken for the purpose of use in ethically approved studies.

At the recruiting centre, samples will be placed in self-sealing bags labeled by interferon trial study number and date of birth only. A histology report will also be placed in the bag, with the name, hospital number and address obscured. Samples will then be mailed securely to the Leeds centre.

9.4. Obtaining clinical data

Information we plan to obtain about the patients from trial data bases will be:

- relevant study number,
- date of birth,
- hospital of recruitment,
- sex,
- date of diagnosis,
- date of randomization,
- depth of primary tumour (Breslow thickness),
- ulceration of primary tumour,
- site of primary tumour,
- number of nodes involved,
- date of primary and lymph node dissection,
- toxicity data (National Cancer Institute Common Toxicity criteria and Karnofsky performance status).

In order to perform RFS/survival analysis, we will also obtain information about:

- recurrence of their disease after randomization (date and type of first recurrence)
- date of death and cause of death or date last known to be alive.

We will also need to know if the patient was randomized to receive interferon or not to assess influence of biomarkers on response to interferon treatment.

This data will be obtained from the clinical trials offices for the 'Aim High' trial and the EORTC data centre and sent via encrypted e-mail. The data will be provided from the study databases labeled by study number and date of birth only and an additional study number will be assigned in Leeds when data sets are complete. The data will be entered onto a Filemaker Pro 8 database and the e-mails from the trial centres will then be deleted. Data obtained from the samples will be fused with the clinical data by clinical trial study number and date of birth only and prior to analysis the identifiers date of birth, date of diagnosis, date of relapse and date of death will be removed and replaced by age at diagnosis, age at relapse and age of death.

9.5. Transfer of samples for analysis

Samples from patients recruited in the UK will be transferred to Leeds for analysis. Samples will be received by our Human Tissue Authority Representative (Mrs. Sandra Tovey) and logged into a central File Maker Pro 8 histopathology database that is currently being developed to comply with the Human Tissue Act 2004. Mrs. Tovey will ensure that no identifiers are retained on the samples or histopathology reports other than study number and date of birth.

Further details of this process are outlined in the 'Standard Operating procedure for Tumour Blocks' – appendix 2 and the 'System Level Security Policy' – appendix 3.

9.6. Pathology review of samples

Primary melanoma samples and nodal samples will be examined using the naked eye to identify blocks containing the most tumour. Where this is not apparent, a section will be taken for H&E staining. These slides will be examined to identify tumour and these areas marked.

Nodes without metastatic deposits and areas of epidermis not involved in the primary

tumour will be identified for germline DNA sampling. Sections of samples and all extracted materials will be labeled with study number and date of birth only.

9.7. Sampling of tissue

From primary tumour blocks and nodal samples with metastatic deposits, unstained sections will be taken for immunohistochemistry and horizontal cores (0.8x2mm) will be taken from the deepest part of the tumour for RNA and DNA extraction. Cores will also be taken to make multi-tumor tissue arrays if sufficient tissue is available.

RNA will be used in expression arrays and extracted DNA will be screened for mutations in key genes associated with melanoma. We aim to collect a minimum of 2 micrograms of RNA and DNA. Pilot studies are currently taking place to optimize RNA collection.

Unstained sections and cores for multi-tumour tissue arrays will be stored for future immunohistochemistry in order to validate markers identified by the gene expression studies.

From lymph nodes without metastatic deposits, multiple sections of tissue will be cut from the samples and germline DNA and RNA extracted from lymphocytes within the nodes. In those cases where lymph node samples are not available germline DNA will be extracted from cores of epidermis not involved in the primary tumour or wide local excision samples. DNA that has been extracted from blood taken in the context of ethically approved research could also be used for this purpose.

9.8. DNA/RNA extraction

From the cores obtained, in the case of primary tumour samples, one core will be used for extraction of RNA using the High Pure paraffin RNA kit (Roche) and Nanodrop will be used to measure RNA concentration and quality. One core will be used for extraction of DNA in batches using the QIAamp DNA micro kit (Qiagen) and Nanodrop will be used to measure DNA concentration and quality. From nodal samples, two cores will be used for RNA and DNA extraction. Additional cores will be used to supplement either the RNA or DNA as required.

Management of tissue blocks is presented in more detail in appendix 2 – Standard Operating Procedure for the Management of Tumour Blocks

9.9. DNA/RNA analysis

From the tumour cells, extracted DNA will be screened for mutations postulated to impact on response to IFN treatment such as: the MxA gene, NK- B, the IFN receptor (IRNAR1 and IFNAR2) and genes in the JAK/STAT signaling pathway. From the tumour RNA, expression arrays for 500-600 genes will be performed using a platform such as the DASL Ilumina platform.

Variation in germline DNA will be explored using SNPs in candidate genes in a platform such as the Ilumina Golden Gate platform. Germline variation exists for many of the genes listed above but other SNPs in immune response genes are also targets. Known functional SNPs will be included as well as tagging SNPs to explain at least 80% of the genetic variation. Germline variation will be tested as determinants of survival overall and in IFN treated patients in survival analysis and determinants of toxicity.

From lymphocyte RNA, expression arrays will be performed.

If DNA extraction proves to be of sufficient quality, and sufficient numbers of samples can be obtained to allow the testing of multiple hypotheses, an additional approach will be to use Illumina chips to perform a genome wide association (GWS) study to identify genes modulating the risk of relapse.

9.10. Data analysis

9.10.1. Primary outcome measure

RFS.

9.10.2. Secondary outcome measure

OS.

The study has four main objectives;

(1) To identify biomarkers that predict response to IFN therapy.

(2) To identify biomarkers that predict development of toxicity from IFN therapy.

(3) To identify prognostic genetic biomarkers, predictive of outcome (RFS, OS) in melanoma patients.

(4) To correlate lymphocyte gene expression profiles with germline variation in

immune response genes.

Variation in germline DNA for candidate genes will be analysed as determinant of OS, RFS and interferon toxicity. Variation in germline DNA, tumour DNA, tumour RNA and lymphocyte RNA expression will be analysed in terms of effect on RFS/OS. Analyses are described in more detail below.

9.11. Pooling data with other centres in Europe

Our aim in Leeds is to collect and analyze samples from patients recruited in the UK to the IFN trials. European samples are being collected by Chemores in collaboration with the EORTC and analyzed in Europe by Professor Eggermont's group in Rotterdam. Any highly significant findings from one data set will be validated in the other data set. In the second phase of the study, anonymised data produced in Leeds will be pooled with data produced by analysis of European samples in collaboration with Chemores and the EORTC. Any significant findings will be validated in another data set in collaboration with Dr. John Kirkwood of ECOG and the University of Pittsburgh, USA.

10. Statistical analysis

10.1 Statistical analysis

10.1.1. Expression array data

Microarray data will be screened using the SAMROC method (Borberg 2003) to identify differentially expressed genes in different groups of patients (relapser/non-relapser, responders to IFN/non-responders etc.). This method uses a penalized t-test that is robust to outliers, minimizes false positive and false negative rates and has good power (Broberg 2003; Kim *et al.* 2006). The concordance between the expression levels from the DASL 3 probes per gene per sample will be assessed and, where appropriate, their average will be used (Fan *et al.* 2004).

10.1.2. Analysis of prognostic biomarkers

For each SNP, evidence for a relationship with RFS or OS will be examined using log rank tests. For biomarkers showing some evidence of a relationship with outcome (SNPs or differentially expressed genes), hazard ratios (HRs) and 95% confidence intervals (CI) will be estimated using single variable and multivariate Cox proportional hazards models, adjusting for other factors previously shown to be of prognostic importance in melanoma (ulceration of the primary tumour, depth of primary tumour (Breslow thickness), number of nodes involved and sex) and for treatment group; the analyses will be stratified by study as in a meta-analysis.

Polymorphisms in linkage disequilibrium within each candidate gene, where there is some evidence of an effect on outcome, will be combined in haplotype analyses.

10.1.3. Analysis of whether biomarkers are predictive of treatment effect and toxicity

For each candidate predictive biomarker, Cox proportional hazards models stratified by study will also be used to estimate HRs and 95% CIs for the effect of treatment group (interferon (IFN) versus observation), separately by each category of the marker, to see whether the HR varies by biomarker status. Interactions between treatment effects and biomarkers will be formally tested using the likelihood ratio test (comparing a survival model including a treatment/marker interaction term and main effects with a model including only main effects). A similar approach will be taken to analyse candidate biomarkers that predict toxicity (defined as myelotoxicity, transaminitis, depression and constitutional). Each factor will be examined individually as will a combined measure of these factors.

10.1.4. Multiple testing

Because of the multiple testing to be carried out in this study, any positive findings will require independent validation. Findings significant at the nominal 5% significance level will be investigated further. As a guide to interpretation of results in the context of multiple testing, false positive report probabilities (FPRP) will be calculated according to Wacholder (2004). The FPRP estimates the risk that a statistically significant interaction is false-positive, based on the observed p-value, the power to detect interaction at that level and the prior probability (PP) of interaction. PP is a subjective measure, used to reflect the strength of the prior hypothesis and preceding data, but applying a range of PPs can test the robustness of any statistically positive findings.

10.1.5. Development of a classification algorithim

In a second approach we will take a more speculative route and attempt to develop a classification rule to identify patients likely to (1) benefit from IFN therapy in terms of survival, (2) develop less toxicity. In this analysis, UK patients will be used to develop the classification rule. Gene expression data and large scale SNP data will be analysed using Random Forests, a classification method that often out-performs most alternatives and avoids overfitting by providing unbiased estimates of predictive performance based on “out-of-bag” samples. If a rule can be developed with good predictive properties, this will be further validated in the non-UK samples. Further, we will examine the prediction of survival and toxicity but the precise approaches will depend upon the pattern of relationship.

Statistical analyses will be conducted using Stata version 10 (StataCorp 2007, College Station, TX).

Statistical analyses will be conducted in Leeds but in conjunction with Professor Wheatley (CTSU, Birmingham) and Dr Stefan Suciu at the EORTC Data Centre.

10.2 Power calculations

Overall, 4173 patients were recruited to these trials as shown below. 1143 patients were recruited in the UK and 3030 in Europe.

A large sample size is required both to make some allowance for the issue of multiple testing in the primary survival analyses and to allow sufficient power to detect interactions between biomarkers and treatment.

Power calculations have been carried out to estimate the power to detect interaction between a biomarker and treatment, using an approximate method (Gonen 2003). Power calculations are based on the assumption that we will be able to extract sufficient DNA and RNA from 90% of tumour blocks from UK patients and 25% from other European patients, based on our previous success rates in the UK and conservative estimates for European samples. This will give just over 1000 UK samples and 750 other European samples from the four trials, and we have assumed a 55% relapse rate. Using all samples there will be 73% power at a 1% significance level and 89% power at a 5% significance level to detect an interaction between treatment with IFN and a biomarker with 50% prevalence, assuming a 50% increase in median relapse-free survival time in patients with the biomarker. By pooling data in this way therefore we anticipate having sufficient power to detect a useful predictor of response at a significance level that will keep the false discovery rate low. Any candidate marker identified in this analysis will require validation in a further data set.

11. Collaboration with EORTC and Chemores

As stated previously UK samples will be collected and analysed in Leeds. Chemores in collaboration with the EORTC will collect samples relevant to this study and analysis will take place in Rotterdam under the supervision of Professor Eggermont. Data from each group will be analysed separately and then pooled and analysed together to increase the power of this study. Any highly significant findings from one data set alone will be validated in the other data set. As part of the collaboration, tissue samples, extracted DNA or RNA, tissue microarrays and clinical data will be exchanged between the groups in a fully anonymised form.

Further details of the Chemores collaboration are available on http://www.chemores.org/.

12. Ethical and regulatory considerations

Ethical approval for the study will be applied for in the UK.

12.1. Approaching potential participants and consent

The patients who took part in these trials did not give informed consent to the use of their stored tissue for research. Both clinical data and tissue samples will be labeled

by study number and date of birth only before they reach the Leeds centre according to the protocol described above. Therefore we plan not to obtain consent for use of data and tissue in this study and have applied to MREC and PIAG for approval to do this.

In order to provide patients with information about the study and give them opportunity to ask questions or opt out of the study, we have developed an information leaflet which we will distribute to the recruiting centres (appendix 4). These will be posted with the relevant clinical trial follow−up correspondence to patients or distributed to patients involved in the relevant clinical trials when they attend clinics.

The information leaflet includes a lay summary of the study, a summary of risks and benefits to the patient if they are involved and details the use of their anonymised tissue samples and data in the future. The leaflet also has contact details for the project coordinator should the patient have any questions or wish to opt out of the study.

The information on this leaflet will also be available on the GenoMel website www.genomel.org. along with the study protocol (for health professionals such as General Practitioners and patients) and contact details for the project coordinator (Dr. Rosalyn Jewell). The format of this web page is currently being discussed with the GenoMel web page designers.

12.2. Risks to patients

Our research involves the use of sensitive patient data and analysis of genetic information. Therefore maintaining confidentiality and data protection issues are of upmost importance and are discussed in detail in the following section.

This study involves no active participation by the patients .

12.3. Benefits to patients

Participants in this study are unlikely to obtain any benefit in terms of their follow-up and future treatment for the duration of the study. However, in the long term, this study could contribute to the identification of genetic markers of survival or response to interferon therapy. This may potentially benefit the patient in the future if they

require further treatment and will benefit patients diagnosed with melanoma in the future.

13. Confidentiality and data handling

In the UK, before transfer to Leeds data and samples will be labeled with study number and date of birth only.

In Leeds, all data will be stored on secure data bases protected by password and data security measures within our organization. There will be limited access to personnel directly involved in the study. No identifiers except study number and date of birth will be used on the databases. Prior to analysis the identifiers date of birth, date of diagnosis, date of relapse and date of death will be removed and replaced by age at diagnosis, age at relapse and age of death.
Information on the 1500 SNPs will be stored in a secure database held in Leeds specifically designed for large scale genetic studies. Security of databases will comply with the departmental and University security policies.

For additional information, please refer to appendix 3 – System Level Security Policy.

14. Duration of study

The proposed start date is September 2008 and the study will last 40 months.

15. Consultation and Review of this Study

This protocol has undergone a process of review by the key groups involved in the collaboration. These include the 'Aim High' group (Professor Hancock, Professor Wheatley), the EORTC group (Professor Eggermont, Dr. Alain Spatz, Dr. Alex Passioukov, Dr. Suciu) and the Nordic group (Dr. Johan Hasson).

Statistical review has been provided by Dr. Jenny Barrett in Leeds, with input from Professor Wheatley and Dr. Suciu (EORTC group).

The protocol has been reviewed by lay members of the Yorkshire Cancer Research Network and National Cancer Research Network. We have also obtained the views of the GenoMel joint advisory group. The protocol has been modified following

suggestions from these groups.

16. Monitoring and audit of study

Regular meetings will be arranged throughout the study to assess progress both within the Leeds research group and with our European collaborators.

17. Funding

Currently, within the Leeds group we have Bramall Fellowship funding which will cover the appointment of the project leader for the duration of the study. The group also has a CR-UK Programme Grant.

We are currently seeking support for a local development of approaches to biomarker discovery in general via the European Research Council Advanced Fellowship scheme. We will submit an application for CR-UK TRICC funding in July 2008.

17.1. Trial sponsorship

The lead sponsor for this research is The University of Leeds.

18. Indemnity

The University of Leeds indemnity arrangements will provide indemnity to the sponsor for any harm to participants arising from the management, design or conduct of this research.

19. Publication Policy

From larger centres which trace tissue blocks themselves, two authors from the centre will be included on publications.

For smaller centres which trace blocks, participating clinicians and colleagues will be acknowledged at the end of publications.

19.1. Dissemination of results

The results of this study will be published in scientific journals and the results will be presented at scientific meetings within the UK, Europe and internationally.

The results of this study will also be published on the GenoMel website (www.genomel.org).

20. Time Line for Study

May 2008	Submission of MREC and PIAG applications
July 2008	Submission of TRICC funding application
September 2008	Start to identify tissue blocks in UK
January 2009	Commence DNA/RNA extraction from UK tissue blocks
January 2009	Ethical and funding applications in Europe
March 2009	Start to identify blocks in Europe
January 2012	Analysis of results
September 2012	Publication of results

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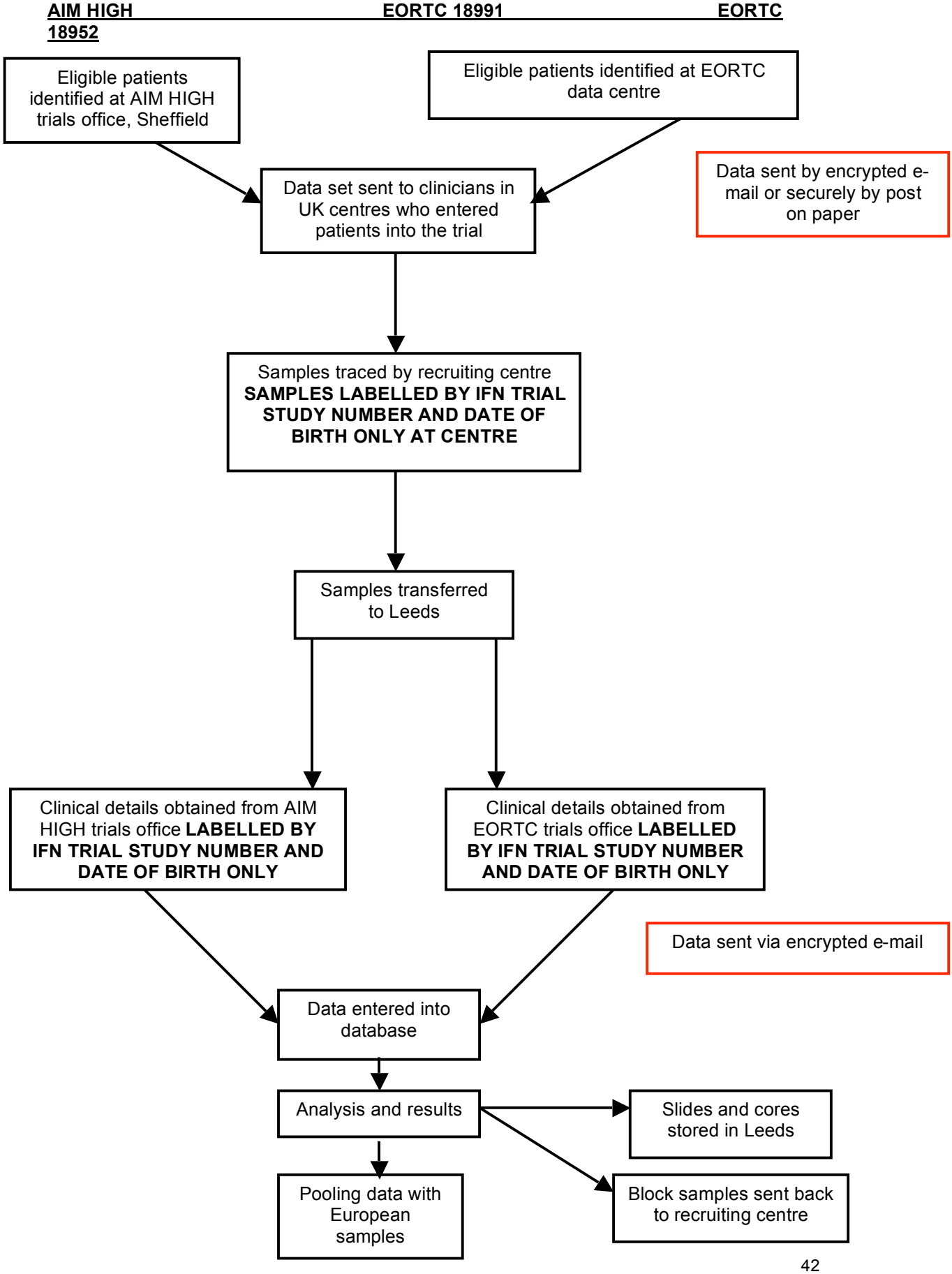
22. Appendices

22.1. Method for collecting data and samples in the UK



22.2. Standard operating procedure for tumour blocks

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Standard Operating Procedure (SOP) for the Management of Tumour Blocks in the Study "Predicting Benefit from Interferon Therapy: Personalized Therapy for Melanoma.

This SOP has been developed in the context of the principles of good clinical laboratory practice (GCLP): some of the requirements of GCLP are described in the study protocol, such as the analytical plan and some are described in the departmental System Level Security Policy Document.

The Sponsor of the trial is the University of Leeds

The PI is Professor Julia Newton Bishop, Cancer Genetics Building, St James's Hospital, Leeds LS97TF j.a.newton-bishop@leeds.ac.uk

The Analytical Project Manager for this project in the laboratory is Dr Juliette Randerson-Moor (J.R.Moor@leeds.ac.uk) and in the analysis group is Mrs Faye Elliott (F.Elliott@leeds.ac.uk)

Date of agreement of the analytical plan June 15th 2008

Aims

To extract normal DNA for studies into hereditary variation which is postulated to modulate survival, response to interferon therapy, and drug toxicity
To extract RNA from normal lymphocytes in nodal samples to understand genetic control of immunological responses to cancer
To extract tumour DNA and RNA to look at mutations or changed expression of genes postulated to affect survival and response to treatment for melanoma patients
To preserve some unstained sections of the tumour to allow immuno-histochemical investigation/ validation of biomarkers of response

Facilities

- Tumour blocks will be received, labeled and archived in the Cancer Genetics Building at St James's Hospital in Leeds. Archived blocks will be documented by Sandra Tovey, using a purpose built data-base, and will be stored in locked metal cabinets in locked rooms.
- Sectioning of the blocks will be carried out in the pathology department of the LIMM building at St James's Hospital in Leeds
- Extraction of RNA and DNA will take place within Lab 1 of the department of Cancer Medicine at St James's Hospital
- Sequencing of DNA will be carried out within the Genome Variation Laboratory at St James's Hospital in Leeds and in Lab 1 in Cancer Medicine.
- Expression arrays will be carried out at Illumina-approved laboratories as yet unspecified.
- If necessary, to meet the scientific aims of the study, samples may be processed in collaborating laboratories.

Procedures

Block Acquisition and Data Management

1. Paraffin embedded tumour blocks will be sought by collaborating centres:
   a. Blocks of the primary melanoma
   b. Wide local excision blocks for stage II melanoma patients.

c. Blocks of nodes removed at block dissection for metastatic melanoma or completion lymphadenectomy after sentinel node biopsy

2. The tumour blocks will be placed into self-sealing plastic bags locally and the bags will be labeled with the clinical trial number and date of birth only. The histology report will be included but the name, address and hospital number of the patient will be obscured.
3. The blocks will be mailed (preferably by secure courier) to Mrs Sandra Tovey at the address below:-

   Mrs Sandra Tovey
   Section of Epidemiology and Biostatistics
   Cancer Genetics Building
   St James's Hospital
   Beckett Street
   Leeds
   LS9 7TF
   UK

4. On receipt, the details of the blocks will be added to:
   a. the laboratory sample management system Pro-Curo (Access based database system) by Sandra. The following information will be recorded:
      i. Study number
      ii. Date of birth
      iii. Date of arrival
      iv. Place the blocks will be stored within the department

      A unique barcode will then be generated which would be affixed to the block and used to track the block around the department. In addition this barcode would also be affixed to any slides, DNA or RNA samples derived from the tissue sampled from the block. Individual blocks from the same individual will be entered onto the database as separate samples and as such will have distinct barcodes
   b. the departmental human tissue data-base (Filemaker Pro 8) by Sandra. She will ensure that no identifiers are retained on the blocks or report other than trial study number and date of birth and she will record the following in the data-base

(i) Study number
(ii) Date of birth
(iii) Hospital of origin of the block
(iv) Histology number of the tissue
(v) Nature of the tissue eg primary tumour, nodal tissue etc
(vi) Number of blocks supplied
(vii) Date of arrival
(viii) Place the blocks will be stored within the department

c. Subsequently this data-base (together with the laboratory Pro-Curo database) will be used to track the block within the department and its ultimate return to the local laboratory (chain of custody)
d. Records of work carried out will be maintained both in laboratory work books (paper) and using the Tissue Data Base.

Management of the primary tumour blocks

1. The blocks and the anonymised histology reports will be examined by Dr Rosalyn Jewell with the naked eye to select those blocks containing most tumour. Where this is not apparent a section of the block will be taken and stained with haematoxylin and eosin. (as detailed in Appendix I sections A and B)
2. Where more than one block of the primary is obtained then up to three containing most tumour will be selected and labeled:-
   a. The test block
   b. The reserve block
   c. A local research block: a block containing tumour which will be returned un-sampled to the local laboratory
3. Where only 1 block is provided then this will be known as the test block.
4. The test block will be sampled as follows:-
   a. 4 unstained slides will be taken on Superfrost glass slides for immunohistochemistry (as detailed in appendix I section A). The need for these samples will be assessed over time as we will compare this to using a core to make a multi-tumour tissue array.
   b. Three horizontal cores will be taken from the deepest part of the tumour and each will be stored separately in sterile 1.5ml microcentrifuge tubes. These

cores will be stored at 4°C prior to extraction of RNA/DNA (as detailed in Appendix I section C).

c. 1 core will be used for extraction of RNA in batches of 24 using the High Pure Paraffin RNA Kit from Roche (as detailed in Appendix I section D) and Nanodrop will be used to measure RNA concentration and quality.
    i. Some will be converted into cDNA (as detailed in Appendix section F)
    ii. The rest will be cryopreserved at -80°C
d. 1 core will be used for extraction of DNA in batches of 24 using the QIAamp DNA Micro Kit from Qiagen (detailed in Appendix I section E) and Nanodrop will be used to measure DNA concentration and quality.
e. The third core will be used to supplement either the RNA or DNA as required.

5. The reserve block will be stored till the end of the project and then returned to the local hospital with the sampled block.
6. Where the patient did not have a nodal recurrence and no germline blood was stored, then normal epidermis in the block will be sampled to obtain normal DNA. Multiple horizontal cores will be taken from the epidermis and DNA extracted as above. Where a wide local excision specimen is available containing no tumour then sections may be used and DNA extracted.

Management of nodal tumour blocks

1. The blocks and the anonymised histology reports will be examined by Dr Rosalyn Jewell with the naked eye to select those blocks containing most tumour. Where this is not apparent a section of the block will be taken and stained with H&E (as detailed in Appendix I sections A and B).
2. Where more than one block of the metastatic tumour is obtained then up to three containing most tumour will be selected and labeled:-
    a. The test nodal block
    b. The reserve nodal block
    c. A local research nodal block: a block containing tumour which will be returned un-sampled to the local laboratory
3. Where only 1 block is provided then this will be known as the test nodal block.
4. The test nodal block will be sampled as follows:-

a. Up to 10 unstained slides will be taken on frosted glass for immunohistochemistry (as detailed in Appendix I section A)
b. Six horizontal cores will be taken from the deepest part of the tumour (as detailed in Appendix I section C) and each will be stored separately in 1.5ml microcentrifuge tubes. These cores will be cryopreserved at 4°C prior to extraction of RNA/DNA.
c. 2 cores will be used for extraction of RNA in batches of 24 using the High Pure paraffin RNA kit from Roche (as detailed in Appendix I section D) and Nanodrop will be used to measure RNA concentration and quality.
    i. Some will be converted into cDNA (as detailed in Appendix section F)
    ii. The rest will be cryopreserved at -80°C
d. 2 cores will be used for extraction of DNA in batches of 24 using the QIAamp DNA micro kit from Qiagen (as detailed in Appendix I section E) and Nanodrop will be used to measure DNA concentration and quality.

5. The reserve block will be stored till the end of the project and then returned to the local hospital with the sampled block.
6. Normal DNA and RNA will be extracted from excised nodes not containing tumour. Multiple sections will be cut from the sample and the whole sample will be extracted.

All samples and materials will be managed in accordance with standard practices in the safe handling of hazardous materials and trial materials.

Implementation of Quality Control

A number of routine quality control measures will be implemented to ensure that data and sample management is maintained. These will vary depending on the nature of the information to be assessed.

1. Block Acquisition

The proportion of samples obtained of those requested for each patient will be used to assess the quality of retrieval.

2. DNA Extraction

The quantity and quality of DNA extracted will be assessed using the Nanodrop, an absorbance based quantification technique, which measure DNA yield and purity. All DNAs will also be subjected to a specially designed fluorescent QC-PCR in which five control amplification products of increasing size (50bp, 100bp, 200bp, 250bp, 300bp) will be amplified. This will give an indication as to the degree of fragmentation of the sample and whether any PCR inhibitors are present in the sample.

3. RNA Extraction

The quality of RNA extraction will be assessed in the first instance using Nanodrop to measure yield and purity. Prior to use in the DASL assay (to be performed by service provider), the quality of RNA samples will be determined with lab-on-chip analysis on the Agilent Bio-Analyser (example given in Appendix II). This will assess quantity of RNA and level of RNA fragmentation in the sample. The DASL assay requires that the average RNA fragment length is ~200 bases. The amplification ability of the RNA will be assessed by a qPCR as part of the DASL assay process.

4. DASL Expression Assays

In order to assess the quality of expression arrays the following quality control measures will be implemented:

a. To monitor intra-assay variation each DASL assay array plate will contain:
   88 test RNA samples
   2 human total RNA reference samples (Stratagene)
   6 technical replicates (a second RNA sample randomly chosen from the 88 test samples on the array)
b. In order to monitor inter-assay variation, a proportion of test samples will be included on two separate assay runs.

Data from each assay will include a quality report generated by the analysis software (BeadStudio, Illumina) which displays graphic control summary for samples based on the performance of controls built in to each assay.

5. Sample Data Management and Results Reporting

The following measures will be implemented to ensure quality of results reporting and data management:

a. The Pro-Curo database incorporates a fully auditable reporting system to track the movement of any sample entered on it. Thus:
    i. Blocks will be signed out of the Pro-Curo Database, prior to any sampling of the block, by the intended user. On completion of sampling the block will be signed back in maintaining the chain of custody.
    ii. All derivative samples (DNA/RNA) associated with a block will be entered onto the Pro-Curo database. Derivative samples will be signed out, by the intended user, in order to perform in-house experiments or send samples to external service providers. On completion of the experiment will be signed back in maintaining the chain of custody.
    iii. Information regarding derivative samples which are used up during the study will remain in the database allowing auditing of all samples irrespective of current status.
b. All results generated will be reported by two independent laboratory personnel.
c. Generated laboratory reports and data files will be checked for accuracy before release to the analysis team.
d. The paper copies of processes used and results generated will be filed in such a way that an audit trail can be carried out.

6. Quality Audit

Internal departmental quality audits will be carried out bi-annually by the melanoma project manager Catherine Plant. These audits will examine the following:

a. The labeling of samples will be audited for accuracy and confidentiality
a. Documentation, QC procedures and reports
b. Review of SOP for sample processing within laboratory

In addition an independent audit will be performed on a yearly basis by the Leeds Institute of Molecular Medicine quality assessor. This individual is not connected to the Section of Biostatistics and Epidemiology and is employed by LIMM to maintain quality control in the GCLP laboratory facilities within LIMM.

Appendix I: Detailed Methodology

A, Sectioning of FFPE tissue blocks using a microtome

1. Set the microtome for 5 micron sections
2. Mount blocks onto microtome with label end facing outwards.
3. Carefully align microtome blade to face of block then using a steady rhythm turn the handle and trim in 5-micron sections until a complete clean face is achieved.
4. Cut required number of 5-micron sections (as defined in study specific procedures) from each block allowing the sections to float on the surface of the microtome water bath.
5. Mount the sections on Superfrost glass slides labeled with study number and barcode.
6. Allow the slide to dry overnight in fume hood.
7. The tissue is fixed by placing the slide on a heat block with tissue side facing up for 20mins.

B, Haematoxylin and Eosin staining of slides

All sections requiring H&E staining are stained using Mayer's Haematoxylin and Eosin. Up to 24 slides may be processed in a single batch. Staining consists of the following steps: de-waxing of the paraffin, rehydration of the tissue through graded alcohols, staining, and dehydration.

1. Place slides in metal slide racks, ensuring tissue direction is consistent.
2. Dewaxing:
    a. Immerse slides in Xylene in a glass slide bath for 5 minutes.
    b. Replace Xylene in the bath and immerse for a further 5 minutes then repeat.
    c. Drain slides on tissue paper
3. Dehydration:
    a. Immerse slides in 100% methanol for 1 min.
    b. Replace methanol and repeat for a further minute.
    c. Transfer slides to baths containing 90% methanol for 1 minute followed by 70% methanol for 1 minute.
    d. Finally place slides under a fast flowing tap for 1 minute.

4. Staining:
    a. Immerse slides in haematoxylin for 3 minutes, then remove to a fresh container and place under a fast flowing tap for 1 minute.
    b. Immerse in Scott's Tap Water for 1 minute then rinse in tap water for 1 minute.
    c. Immerse slides in eosin stain for 3 minutes.
    d. Rinse slides by flushing under a fast tap for 30 seconds and allow racks to drain on tissue paper.
5. Rehydration:
    a. Immerse slides in 70% methanol for 1 min.
    b. Transfer slides to baths containing 90% methanol for 1 minute followed by 100% methanol for 1 minute.
    c. Replace methanol and repeat for a further minute.
    d. Immerse slides in xylene for 1-5 minutes to ensure section is firmly fixed to slide.
6. Mount slides by placing 1 drop of depex onto a glass coverslip. Place slide, tissue side down, onto the coverslip starting at one edge and taking care to prevent air bubbles forming between slide and cover slip.
7. Dry slides overnight in fumehood prior to review

C, Slide review and tumour sampling.

H&E slides will be reviewed for tumour content and an area of the tumour selected for sampling. The area selected would ideally be at the deepest part of the tumour possible whilst still containing a high percentage of tumour cells and minimal stromal invasion. The ideal spot for sampling will be marked on the H&E slide using a fine tip permanent marker.

Sampling of the tumour will be performed using Tumour Micro Array (TMA) Building Apparatus. As the needle on the TMA is not disposable and cannot be cleaned using standard alcohol based techniques, between blocks, one core will be taken from a blank paraffin block, to effectively clear the needle of any potential contaminating tissue between sampling.

1. Secure the block to be sampled in the rig.

2. Orientate and place the marked H&E slide over the tumour block.
3. Align the Tumour Micro-Array (TMA) needle above the marker spot.
4. Remove the slide and manually guide the needle down to the block.
5. Remove a 0.8 x 2mm core from the block and placed in a labeled 1.5ml microcentrifuge tube. Store at 4°C prior to processing.

D, RNA extraction from FFPE tissue using Roche High Pure RNA Paraffin Kit

RNA will be prepared initially in a tissue culture hood designated for RNA preparation to ensure that all surfaces, tips and designated pipettes can be maintained as RNase-free. It is envisaged that RNA preparation will move to a designated RNA preparation room when completed (estimate 3-6 months). All RNA extractions will be given a unique consecutive RNA extraction number of the format RYY/0000 (eg. R08/1234) prior to starting extraction and this number will be used to label all consumables used during the extraction process. Final eluted RNA sample will be labeled with RNA extraction number, Study number and date of extraction.

Total RNA is prepared from FFPE tissue using the High Pure RNA Paraffin Kit (Roche, Cat No 03 270 289 001) using a modified protocol as recommended by Illumina for use with Illumina's DASL technology.

1. Dewaxing (to take place in the fume cupboard, spray pipettes with RNase free spray before use):
    a. Add 500µl xylene to 1 FFPE core in a 1.5ml microcentrifuge tube and pulse vortex for 10 seconds. Heat samples at 45^{o}C for 15 minutes and then pulse vortex for 10 seconds. Carefully remove xylene by pipetting.
    b. Add 400µl absolute ethanol, invert and pulse vortex to mix. Carefully remove ethanol by pipetting.
    c. Add 400µl of absolute ethanol, invert and pulse vortex to mix, then carefully remove ethanol by pipetting.
    d. Blot tube on paper towel to remove excess ethanol
    e. Dry the tissue pellet in a heating block set at 45°C for 5 minutes

2. Tissue digestion (all of the following steps to take place in the RNA preparation hood):
    a. Add 100µl supplied tissue lysis buffer, 16µl 10% SDS and 40µl supplied Proteinase K to the dry tissue pellet.
    b. Vortex and incubate at 55°C for a maximum of 4 days, repeating vortex at regular intervals until no remnant of the core remains in solution. **It is essential to ensure that the core remains in solution and not stuck to the side of the 1.5ml tube during the incubation period.**
3. Add 325µl supplied Binding Buffer and 325µl absolute ethanol to homogenized tissue solution and mix by gentle pipetting.
4. Add lysate to upper reservoir of a labeled High Filter tube inserted into a labeled collection tube. Centrifuge at 8,000*g* for 30 seconds.
5. Discard flow through liquid from collection tube. Centrifuge at maximum speed (12,000-16,000*g*) for 30 seconds to dry filter.
6. Wash bound nucleic acids:
    a. Add 500µl supplied Wash Buffer 1 to the upper reservoir of the filter tube. Centrifuge at 8000*g* for 15 seconds. Discard the flow through liquid.
    b. Add 500µl supplied Wash Buffer 2 to the upper reservoir of the filter tube. Centrifuge at 8000*g* for 15 seconds. Discard the flow through liquid.
    c. Add a further 300µl of Wash Buffer 2 and centrifuge at 8000*g* for 15 seconds. Discard the flow through liquid.
    d. Centrifuge at maximum speed (12,000-16,000*g*) for 2 minutes to ensure the removal of residual wash buffer.

Although the column is optimised for RNA isolation, some DNA may still be present in the filter so an extra step of DNA digestion using DNase I is integrated into this protocol to ensure a pure RNA only yield. This can be important for some downstream applications (such as the DASL assay) that involve copy number measurement of RNA after conversion to cDNA.

7. Removal of contaminating DNA:

a. Remove filter column to a labeled sterile 1.5ml microcentrifuge tube. Add 90µl of supplied Elution Buffer to the centre of the upper reservoir of the filter column. Centrifuge at 8,000*g* for 1 minute.
b. Add 10µl supplied DNase Incubation Buffer and 1µl supplied DNase I to the eluate. Pulse centrifuge. Mix by gently by tapping or vortexing. Incubate for 45 minutes on a heating block at 37°C. Pulse centrifuge again to collect droplets from the lid.

8. Repeat steps 2 to 6 inclusive with the following alteration:
   a. In step 2 add 20µl Tissue Lysis Buffer and 18µL 10% SDS and incubate at 55°C for 1 hour in a waterbath or heating block.
9. Elute pure RNA:
   a. Insert filter column into sterile 1.5ml microcentrifuge tube.
   b. Add 26µl RNase-free diH_2O carefully to the centre of the filter column. Incubate at room temperature for 5 minutes.
   c. Centrifuge at 8,000*g* for 1 minute. Remove filter column.
10. Centrifuge eluate at maximum speed (12,000-14,000*g*) for 2 minutes to pellet any contaminating residual glass fibres carried over from the filter. Carefully remove supernatant into a 0.2ml strip PCR tube (tube 1) and then aliquot 6µL of this supernatant into another strip PCR tube (tube 2) for cDNA production and nanodrop analysis.
11. Use 1.5µL (from tube 2) to quantify amount of RNA extracted using Nanodrop.
12. If quantity of extracted RNA is insufficient (<20ng/µL RNA – at least 200ng RNA is needed for the DASL assay), extract second core of tissue.
13. Store eluted RNA (tube 1 and 2) for cDNA production and analysis at -80°C until required.

E, DNA Extraction from FFPE tissue using Qiagen QIAMP DNA mini kit

DNA will be prepared in designated DNA preparation area within the main laboratory. All DNA extractions will be given a unique consecutive DNA extraction number of the format YY/0000 (eg. 08/1234) prior to starting extraction and this number will be used to label all consumables used during the extraction process. Final

eluted DNA sample will be labeled with DNA extraction number, Study number, date of extraction and barcode.

DNA is prepared from FFPE tissue using the QIAmp Kit (Qiagen, Cat. No. 51306) using a modified protocol for tissue cores.

1. Dewaxing:
    a. Place 1-2 cores in a 1.5ml microcentrifuge tube. Add 1ml xylene and incubate at 37°C for 30 minutes. Pulse vortex the sample at regular intervals.
    b. Carefully remove xylene using a pipette. Add 1ml absolute ethanol to the tube and pulse vortex to remove residual xylene.
    c. Carefully remove ethanol using a pipette. Add 1ml 70% ethanol, pulse vortex and completely remove ethanol by pipetting.
    d. Evaporate residual ethanol from the cores by placing open tubes on a heating block for 5-10 minutes at 37°C.
2. Cell Lysis:
    a. Add 180µl supplied Buffer ATL and 20µl supplied Proteinase K solution. Vortex to mix ensuring core is submerged in buffer on completion.
    b. Incubate in a waterbath set at 56°C for 48-72 hours until there is complete lysis of the sample (i.e. until no remnant of core remains in solution). Vortex at regular intervals to mix. Pulse centrifuge tubes to remove and liquid from lid of tube.
    c. Add 200µl supplied Buffer AL to sample. Pulse vortex for 15 seconds to mix. Incubate in a heating block set at 70°C for 10 minutes. Pulse centrifuge tubes to remove and liquid from lid of tube.
    d. Add 200µl absolute ethanol to sample. Pulse vortex for 15 seconds to mix. Pulse centrifuge tubes to remove and liquid from lid of tube.
3. Insert a QIAamp Spin Column into the top of a 2ml collection tube (both supplied). Carefully apply the sample to the spin column ensuring that no liquid touches the rim of the column. Centrifuge at 6000*g* for 1 minute.
4. Washing of bound nucleotides:

a. Place spin column in fresh collection tube. Add 500µl Buffer AW1. Centrifuge at 6000*g* for 1 minute.
b. Place spin column in fresh collection tube. Add 500µl Buffer AW2. Centrifuge at 16,000*g* for 3 minutes. Discard flow through liquid.
c. Centrifuge at 16,000*g* for 1 min to prevent buffer carryover into the elution step.

5. Elution of DNA:
    a. Place spin column in a sterile 1.5ml microcentrifuge tube (it is necessary to remove the lid of the microcentrifuge tube to ensure the spin column can sit comfortably within the centrifuge).
    b. Add 20-30µl sterile molecular biology grade ddH_2O to the upper reservoir of the spin column. Incubate at room temperature for 1 hour.
    c. Centrifuge at 6,000*g* for 1 minute to elute DNA. Transfer eluate to a sterile 1.5ml microcentrifuge tube.
6. Quantify DNA extracted using Nanodrop.
7. If insufficient DNA extracted (exact quantity depends on analysis, 25ng for each PCR and 100ng for MLPA), extract DNA from a second core of tissue.
8. Store DNA at 4°C until required.

F. Production of cDNA using Invitrogen Superscript ™ First-strand synthesis system.

Production of cDNA will be carried out using the Superscript ™ First-strand synthesis system (Invitrogen, Cat. No. 11904-018). The kit protocol for first strand synthesis uses random hexamer primers. This method of priming is used for cDNA production for the DASL assay performed externally. A modified version of the supplied protocol will be used optimized for use with low concentration FFPE RNA. For quality control purposes negative and positive controls will be included in each experiment. The negative control will consist of test RNA but no reverse transcriptase and the positive control will consist of control RNA provided with the kit. All heating steps will be performed on a Applied Biosystems 9700 Thermal Cycler.

1. First strand synthesis

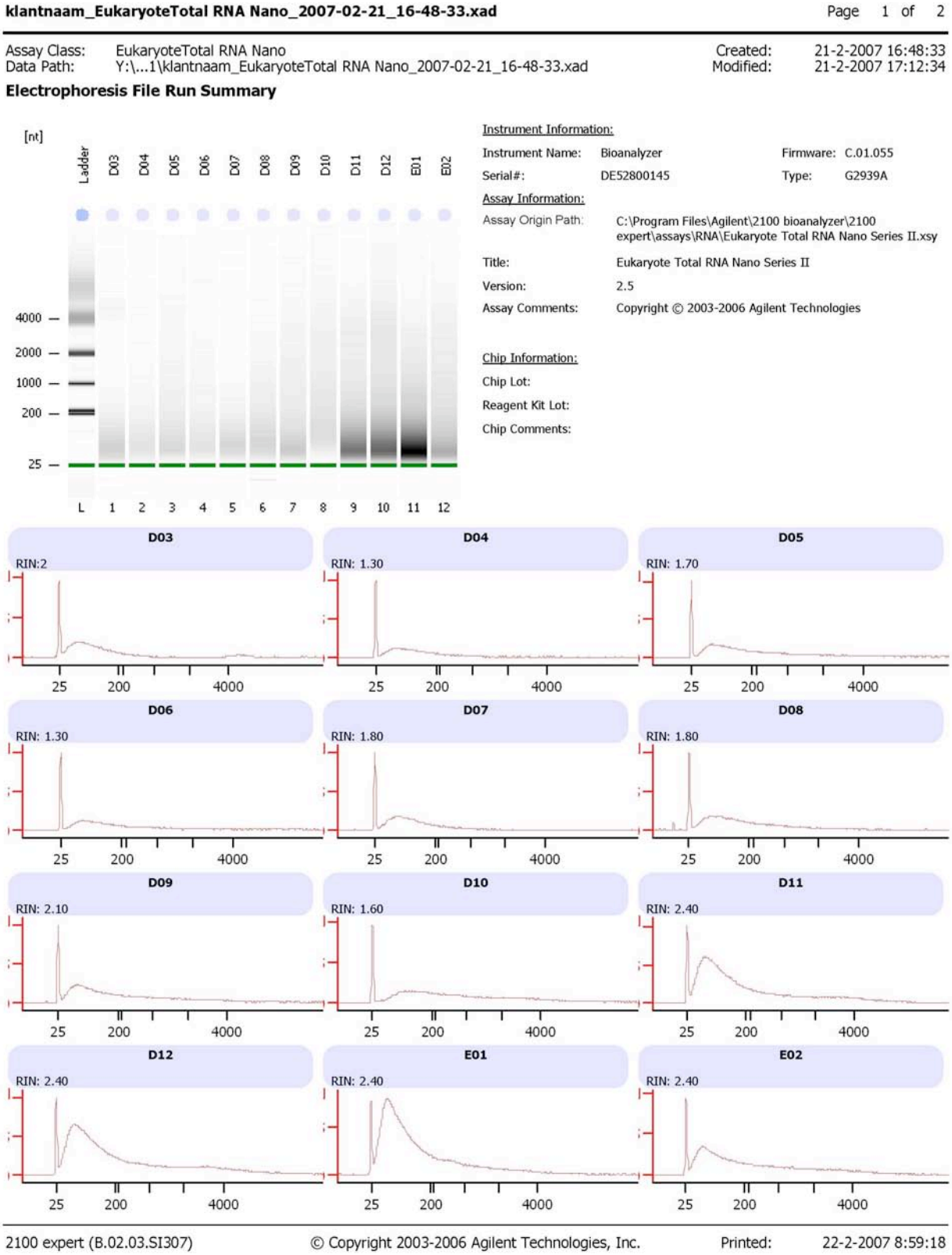
a. Add 5µL RNA (10ng/µL to 1000ng/µL) to a 0.2ml microcentrifuge tube. Add 4µl of 10ng/µl supplied random hexamer primers and 1µl 10mM dNTP mix. Incubate at 65°C for 5 minutes.
b. Remove to ice while the following mastermix is prepared in a sterile 1.5ml microcentrifuge tube. The total volume should be calculated for n+3 samples (where n is the number of test samples not including controls).

Supplied Reagent	**Volume /rxn (µl)**
10X RT buffer	2
25mM $MgCl_2$	4
0.1M DDT	2
RNaseOUT Recombinant Ribonuclease Inhibitor	1
Total volume	9

c. Add 9µl of mastermix to the RNA/Primer mixture on ice. Vortex briefly to mix and pulse centrifuge at 12,000-14,000*g* to collect liquid.
d. Incubate tubes at 25°C for 2 minutes. Add 1µl of supplied Superscript II Reverse Transcriptase to each tube except the negative control. Incubate at 25°C for a further 10 minutes
e. Incubate tubes at 42°C for 50 minutes followed by 70°C for 15 minutes to terminate first strand synthesis. Place tubes on ice.

2. Removal of residual RNA.
    a. Add 1µl supplied RNase H to each tube. Incubate at 37°C for 20 minutes. Store cDNA at 4°C until required.

Appendix II: Example of lab-on-chip analysis report of RNA on the Agilent Bio-Analyser.



22.3 System level security policy

University of Leeds Faculty of Medicine and Health
Section of Epidemiology & Biostatistics
Leeds Institute of Molecular Medicine

'Predicting Benefit from Interferon Treatment in Melanoma' Research Study

System Level Security Policy

1. INTRODUCTION

This system level security policy (SLSP) document is for a research study, 'Predicting Benefit from Interferon Treatment in Melanoma', that will be conducted in the Section of Epidemiology & Biostatistics, Leeds Institute of Molecular Medicine (LIMM), Faculty ofMedicine and Health, at the University of Leeds. The Section is based on the St. James's University Hospital (SJUH) campus site of the University.

This study (hereafter referred to as the 'Interferon Study') plans to use clinical data from three previously conducted randomized controlled trials (RCTs) which recruited patients with melanoma to assess the benefit of interferon. Biological samples that were collected, outwith the RCTs and during the diagnostic and therapeutic process or during other ethically approved research, will also be identified for these patients. Genetic data will be created from genetic tests on the biological samples. The tests will examine whether genetic alterations predict survival in melanoma, benefit from interferon treatment and development of side effects with interferon.

This document outlines how non-consented patient identifiable data will be held securely within the Interferon Study. Points that are still to be confirmed are highlighted in italics.

2. SCOPE

Patient identifiable data will be used by the Data Manager and Data Users within the Faculty of Medicine and Health. See Table 1 for details of these post holders and their responsibilities.

Data will be the scope of this policy at the point that it is transferred into the safekeeping of the Data Users. The transfer of data and all subsequent handling by the Data Users is covered by this SLSP. Data will be transferred to a secure storage area on the University of Leeds computer network by the Data Users. This SLSP covers the transfer of data to the databases and the storage, management and analysis of the data in the University of Leeds until the end of the Interferon Study. This process is outlined in '6. DATA'.

The SLSP, the PhD student and University of Leeds staff working on this project, and the server and computers used in the Interferon Study that are on the University of Leeds network are all subject to the following policies:

- Code of Practice on Data Protection, published June 2003;
- Information Security Policy, April 2008 version;
- All policies that support the Information Security Policy, e.g. the Mobile and Remote Working Policy, April 2008 version.

The University's code of practice on data protection accords with the Data Protection Act 1998 and takes into account the codes of practice published periodically by the Information Commissioner's Office. The University's code forms part of the formal contract of employment for staff and part of the formal agreement between students and the University. Staff, and where appropriate, students must abide by the code and any failure to comply with it could result in disciplinary proceedings.

The Information Security Policy has been compiled jointly by consultants from KPMG (the University's Auditors) and the University's IT Security Co-ordinator. It is based upon the International Standard ISO 17799 (BS 7799) The Code of Practice for Information Security Management.

The Interferon Study involves the collection, transfer, analysis and storage of highly confidential personal data and this SLSP is designed to meet or exceed all the requirements of the University's Information Security Policy.

3. SYSTEM MANAGEMENT

See Table 1 for roles and responsibilities of individuals involved in system management of the Interferon Study.

The implementation of this SLSP will be initiated at a meeting of all Interferon Study members, based at the University of Leeds, prior to the start of data collection. The purpose of this meeting will be to ensure that all members of the Interferon Study:

- Are aware of their responsibilities for data security within the SLSP;
- Understand the measures being taken and the reasons for them;
- Understand the procedures contained within the SLSP and are able to follow them.

Table 1: System Management – Roles and Responsibilities

Role	**Name**	**Responsibility**	**Access to confidential data**
IT Manager	Mr Andy Pellow, IM&T manager, University of Leeds, Faculty of Medicine and Health, Worsley Building, Clarendon Way, Leeds, LS2 9NL	Data security with the Faculty of Medicine and Health	No
System Managers and Server Managers	Mr Paul McGrath, IT manager, Mr Geoff Cross, IT Systems Administrator (MacIntosh), Dr David Perkins, Principal Research Scientist (Section of Oncology and Clinical Research), LIMM, Wellcome Trust Brenner Building, SJUH, Beckett Street, Leeds, LS9 7TF	Implementation of technical aspects of SLSP and server management: • Maintenance of server hardware and software; • Backup of server data.	No
Data Manager	Ms May Chan, Database Development Manager, LIMM, Section of Epidemiology & Biostatistics, Cancer Genetics Building, SJUH, LS9 7TF	Implementation of data management aspects of SLSP	Yes
Data Users	All from Section of Epidemiology and Biostatistics: Dr Rosalyn Jewell (Interferon Study Project Manager/PhD student) Professor Julia Newton-Bishop (Chief Investigator/PhD supervisor) Professor D Timothy Bishop (Section leader/PhD supervisor) Dr Jenny Barrett (Reader in Biostatistics and Genetic Epidemiology/PhD supervisor) Mrs Faye Elliott (Statistician) Dr Ruby Chang (Statistician) Ms Sandra Tovey (Section's Human Tissue Act lead)	Collection, inputting, transfer and analysis of data	Yes

	Ms May Chan (Database Development Manager) Dr Catherine Plant (Melanoma Group Project Manager) **Laboratory staff** Dr Juliette Randerson-Moor (Laboratory Manager) Dr Mark Harland (Molecular Biologist) Ms Samira Lobo (Laboratory technician) Ms Kairen Kukalizch (Molecular Geneticist) Laboratory technician (to be appointed)		
Information Custodian	Dr Catherine Plant	Overall implementation of SLSP	Yes

4. COMPUTER HARDWARE

Table 2 provides details of the servers and Table 3 provides details of the computers that will be used in the Interferon Study.

Table 2: Details of servers that will be used in the Interferon Study

Server name	Software	Manager
fmpro1.leeds.ac.uk	FileMaker Pro (FMPro) 8	Geoff Cross
fmpro2.leeds.ac.uk	FMPro 9 advanced	Geoff Cross
xserve1.leeds.ac.uk	10.4 tiger server	Geoff Cross
CRUK 2003	Windows 2003 server	Paul McGrath
bcos.leeds.ac.uk	RedHat EL 5	David Perkins
bcos2.leeds.ac.uk	RedHat EL 5	David Perkins

Table 3: Details of computers that will be used in the Interferon Study

Name	Computer model	System Version
May Chan	Optiplex 745 (desktop)	XPSP3
Rosalyn Jewell	MacBook (laptop)	Mac OS X 10.5.2
Julia Newton-Bishop	MacBook (laptop)	Mac OS X 10.5.2
Tim Bishop	MacBook Pro 15” (laptop)	Mac OS X 10.5.2
Jenny Barrett	EVO (desktop)	Windows 2000
	Notebook V8010 (laptop)	XPSP3
Faye Elliott	dc7600s (desktop)	XPSP3
	Notebook 3613WLH (laptop)	XPSP3
Ruby Chang	MacBook Pro 5” (Pc/Mac laptop)	Mac OS X 10.4.10
Sandra Tovey	MacBook (laptop)	Mac OS X 10.4.11
Juliette Randerson-Moor	Dell Latitude D620 (laptop)	XPSP3
Kairen Kukalizch	MacBook 13” (PC/Mac laptop)	XPSP3
Catherine Plant	MacBook Air (laptop)	Mac OS X 10.5.2
Communal laboratory computer	iMac (desktop)	Mac OS X 10.4.10
Laboratory technician	To be assigned	To be assigned

5. COMPUTER SOFTWARE

For server and Data Users computer operating system software see Tables 2 and 3, respectively. Updated versions of all software will be installed as they become available.

a) Virus software
Each computer has Network Associates McAfee VirusScan enterprise (minimum v7.0) configured to auto-update weekly from the Information Systems Services anti-virus server or directly from the internet.

b) Encryption software
The Macs use Disk Utility to create encrypted disk images on laptop hard drives. On Macs running Mac OS X 10.4 128-bit encryption is used with 256-bit encryption being available for Macs running Mac OS X 10.5. Data Users are asked to use passwords that are 12 or more characters long and which comply to FIPS-181 (The U.S. Department of Commerce password generation standard). This software is used by the Section of Epidemiology and Biostatistics to encrypt memory sticks although it is not envisaged that such removable media will be used for the Interferon Study.

The Section’s computing department is reviewing the encryption of both Macs and Windows-based machines and will shortly be trialing the SecuriKey system (http://www.securikey.com). The trial

should be completed by September 2008 and if successful this system will be rolled out to both Mac and Windows-based machine users.

c) Email software
MS Entourage. *To confirm how email attachments will be encrypted by Data Users; this process is currently under review and it is not envisaged that there will be an immediate need for Data Users to email data to external collaborators.*

d) Database software
FMPro 8 and MS Access

e) Laboratory software
MS Excel, CodonCodeAligner (sequencing), GeneMarker v.1.7 (genotyping), SDS v.2.1 (genotyping (Taqman) and gene expression), BeadStudio (gene expression)

f) Statistical software
Stata v.10.0, SAS and BC gene

6. DATA

The data that will be held by the Interferon Study and how it will be managed is described below.

The three previously conducted RCTs from which the Interferon Study will use UK data collected from trial participants are:

- The 'AIM HIGH' trial (Hancock *et al.*, 2004);
- The 'EORTC[1] 18952' trial (Eggermont *et al.*, 2005);
- The 'EORTC 18991' trial (Eggermont *et al.*, 2007).

These patients were recruited to the trials by clinicians based at one of twenty-five local clinical centres.

a) Biological samples
Patients will be identified to each of the local clinical centres as potential participants to the Interferon Study as follows:

- 'AIM HIGH' patients will be identified from the trial database by staff at the trial's office in Sheffield extracting the following patient data:
    - name;
    - date of birth;
    - hospital of recruitment;
    - hospital number;
    - date of randomization;
    - RCT study number.
- EORTC trials' patients will be identified from the trail database by staff at the EORTC data centre in Brussels extracting the following patient data:
    - initials of name;
    - date of birth;
    - hospital of recruitment;
    - hospital number;
    - date of randomization;
    - RCT study number.

These data will be sent either via paper copy in secure post or encrypted email to the clinicians in the UK clinical centres who entered patients into the RCT.

Tissue samples
The local centre will then trace the relevant tissue samples which are currently stored in the local pathology laboratories. The tissue samples include melanoma tumours, wide local excisions and

[1] European Organisation for Research and Treatment of Cancer

lymph nodes. Clinicians will place the tissue samples in a self-sealing plastic bag and label the bag with RCT number and date of birth; these samples will already be labeled with a local laboratory sample number. Clinicians will obtain a copy of the histopathology report for the tissue samples, and will obscure the patient's name, address and hospital number on this report.

The samples and reports will be couriered to Sandra Tovey, a Data User, in compliance with UN3373 regulations, as appropriate. If samples are sent through the regular post they willbe marked 'PATHOLOGICAL SPECIMEN – FRAGILE – HANDLE WITH CARE'.

The tissue samples will be logged by Sandra in a central FMPro 8 histopathology database that is being developed currently to comply with the Human Tissue Act 2004. The following data will be recorded in the database using data on the samples and the accompanying histopathology report:

- RCT Study number;
- Date of birth;
- Study name='Interferon Study';
- Hospital of origin of the block;
- Histology number of the tissue;
- Nature of the tissue, e.g. primary tumour, nodal tissue etc;
- Number of blocks received;
- Date of receipt;
- The location of the block at all times.

Extracted germline DNA samples
Germline DNA samples, extracted from blood samples, will be retrieved for those patients that also participated previously in other ethically approved research. This research was conducted by the clinical centres at Leeds and Glasgow and other clinical centres may confirm at a later date that they will participate. The Leeds samples are currently labeled with aunique research study number, date of birth, DNA extraction number, date taken, date processed and a barcode number and will not be relabeled for the Interferon Study. The other local centres will label the samples with RCT number and date of birth; *it is to be confirmed how these samples are currently labeled. It is to be confirmed whether any other data will accompany the germline DNA samples although the other clinical centres will be asked not to send any data with the germline DNA samples that enablethe Data Users to identify an individual.*

Samples from outside Leeds will be sent to Juliette Randerson-Moor, a Data User, by courier or post as described for the tissue samples above. The samples will be logged on an Excel spreadsheet that is used to log all of the extracted DNA samples in the Section of Epidemiology and Biostatistics' research studies.

Germline DNA samples are also logged on an Access DNA database by the Data Users based in the laboratory. This database is used to record all of the biological samples that the Section's laboratory group possesses. The following data will be entered into the database for those samples received from outside Leeds:

- RCT study number;
- Date of birth;
- Study name='Interferon Study';
- Study code;
- Study cancer type='melanoma';
- Any other laboratory processing dates/numbers.

New samples
The FMPro histopathology database will be used to create a list of tissue samples from which new samples will be created. *What data are included on the list and whether it is in electronic and/or paper copy format is to be confirmed as this database is still in development.*

The following new samples will be created from the original tissue samples by the Data Users based in the laboratory:

- Germline DNA samples - will be extracted from tissue removed from lymph nodes without metastatic deposits and, where this is not possible, tissue from wide local excision samples will be used;
- Tumour RNA samples – will be extracted from tissue removed from the primary tumour samples and/or metastatic deposits in lymph node samples;
- Tumour DNA samples – will be extracted from tissue removed from the primary tumour samples and/or metastatic deposits in lymph node samples;
- Slides each containing a section of tissue from either the primary tumour samples or lymph node samples containing metastatic tumour.

The germline and tumour DNA samples will be logged on the Excel DNA spreadsheet described above. The tumour RNA samples will be logged on a separate Excel spreadsheet and will contain similar data to the DNA spreadsheet. All of these samples will be logged in the Access DNA database. The slides created from the blocks will be logged in the FMPro histopathology database.

Tissue microarrays will be created from the tumour RNA samples and will be logged in an Excel spreadsheet.

All of the above new samples will be labeled with:

- RCT study number;
- Date of birth;
- Laboratory processing dates/numbers.

Slides will be sent for pathological review to the consultant histopathologists Professor Martin Cook at the Royal Surrey Hospital in Guildford, and Drs Will Merchant and Sara Edward who are based at SJUH. Sandra Tovey will deliver slides to Will and Sara by hand. Slides will be sent to Martin Cook as described for the tissue samples above.

b) Laboratory genetic tests

The Access DNA database will be used to create an Excel spreadsheet and hard copy list of samples to be used in laboratory tests. The list will contain RCT study number, date of birth and extraction number. The electronic list is stored for future reference. The hard copy list may be stuck into a Data User's laboratory book.

Data from most laboratory tests do not contain identifiers, but this depends on the software that is outputting the data and study number may be included in the output. Similarly, laboratory results in laboratory books may contain study number data, but with no other personal identifiers.

Individual tumour RNA samples will be sent to Illumina-approved laboratories who will create expression arrays for testing. The only identifier to be sent with these samples is RCT study number. Samples will be sent to these laboratories by courier as described for the tissue samples above.

Excel data sets will be stored in a restricted area on a server. However if laboratory testsgenerate very high volumes of data, e.g. single nucleotide polymorphism (SNP) data, then these data will be stored on the BC gene database. *It is to be confirmed whether any clinical data will be uploaded to this server for statistical analysis*; age, sex and recruitment centre data have been uploaded for other research studies in the Section, for example.

Any blocks of tissue remaining after the laboratory tests will be sent back to the recruiting clinical centre after the study has ended (in approximately February 2012) although tissue removed from blocks, including slides created, cores and extracted DNA/RNA, will be retained for up to 20 years.

c) Clinical data

The AIM HIGH and EORTC offices will supply the Data Users with the following clinical datafrom the trial databases:

- RCT number;
- Date of birth;
- Hospital of recruitment;
- Sex;

- Date of diagnosis;
- Date of randomization to trial;
- Depth of primary tumour (Breslow thickness);
- Ulceration of primary tumour;
- Site of primary tumour;
- Number of nodes involved;
- Date of primary and lymph node dissection;
- Toxicity data (National Cancer Institute Toxicity Criteria and Karnosfsky performance status);
- Date(s) and type(s) of relapse after randomization (if applicable);
- Date of death and cause of death (if applicable);
- Date last known to be alive;
- Randomized to receive interferon or not in trial.

The trial offices will send the data file as an encrypted email attachment which will be deleted once the data are entered onto the FMPro 8 Interferon Study database that will be created solely for the Interferon Study.

d) Statistical analysis

Excel data and FMPro data will be converted to Stata and SAS data sets for statistical analysis. During creation of data sets, date of birth data, date of diagnosis data, date of relapse data and date of death data will be removed and replaced with age, age at diagnosis, age at relapse and age at death fields.

Statistical analysis of the UK data will be conducted in Leeds in conjunction with Professor Keith Wheatley at the Clinical Trials Unit at the University of Birmingham and Dr Suciu at the EORTC data centre in Brussels. Data will be linked-anonymised, using the RCT study number as an identifier, and will be emailed in an encrypted attachment.

e) Data sharing

CHEMORES, an European Union funded research collaboration involving eight European countries, in collaboration with the EORTC are currently collecting samples relevant to this study and plan to undertake analysis of these samples in Europe (www.chemores.org). It is anticipated that extracted DNA and RNA samples, tissue microarrays, clinical and genetic data may be exchanged between the CHEMORES collaborators Professor Alexander Eggermont at the Erasmus University in Rotterdam and Dr Johan Hansson at the Karolinska Institutet in Stockholm.

The samples and data sent to Europe will be labeled by RCT study number only, i.e. the identifier date of birth will be removed. We do not expect the biological samples will be returned to Leeds as the samples that would be sent would only be sufficient to run a laboratory test.

A pooled analysis of the UK and European data will take place in Leeds.

7. RISK ASSESSMENT

A risk assessment has been carried out by the Information Custodian walking through the data management process described (6. DATA) to identify potential areas of risk during the movement and processing of data (Table 4).

Table 4: Risk Assessment

Risk	Description	Impact	Likelihood	Actions taken to minimise risk
Breach of confidentiality	Samples arrive in Leeds with identifiers, e.g. names, attached	High	Low to medium	Ms Sandra Tovey, the Human Tissue Act lead in the Section of Epidemiology and Biostatistics, will check all samples and remove any identifiers, other than RCT number and date of birth, before handing them to the other Data Users
	Local pathology laboratory needs assistance to locate tissue samples	High	Low to medium	Dr Rosalyn Jewell will visit the laboratory to provide assistance
	Loss or theft of laptop	High	Low	Any confidential data will be stored in a password protected encrypted space on laptop hard drive
	Loss or theft of histopathology reports Theft of servers Deliberate "hacking" into server Accidental disclosure of information	High	Low	See sections on data security and access control Data Users only will have access to patient names in the exceptional circumstances described above in the table
User Authentication	Disclosure of user ID/Password Disclosure of user name and password to encrypted space	High	Low	See section on access control
Loss of data	Loss of server or paper data through fire, theft, flood, accidental or deliberate damage, hardware failure Loss of data through software failure	Medium	Low	See section on data backup
Failure of the network	Local network failure restricted to project or university wide campus network failure	Low	Low	See sections on Business Contingency and Disaster Recovery
Failure of the hardware	Server or laptop hardware failure preventing access to data but not destroying data	Low	Low	See sections on Business Contingency and Disaster Recovery

Failure of the software	Software failure preventing access to data but not destroying data	Low	Low	See sections on Business Contingency and Disaster Recovery

8. DATA PROTECTION

Patient identifiable information is needed solely for the purposes described and will not be further processed in any form. Only linked-anonymised information will be removed from the database for analysis and sharing. Only anonymous information will be published. All patient identifiable information will remain securely stored on the databases in accordance with the SLSP for the duration of the project.

The Faculty of Medicine and Health has Data Protection Registration through the University of Leeds. The registration number is Z553814X. The proposed use of patient identifiable information must satisfy the requirements of the Data Protection Act 1998 (see Section 60 application for further details).

9. DATA SECURITY

a) Security of paper data
Patient details sent from trial offices to clinical centres in paper format will be securely destroyed by the clinical centres once the relevant patient samples and histopathology reports have been located.

The labeled tissue samples and histopathology reports sent to Sandra Tovey will be stored in a locked cabinet in the Cancer Genetics Building. Access to the keys will be restricted to the Data Users.

Other biological samples are stored in the Cancer Medicine Research Unit building at SJUH. *The security of biological samples used in the laboratory in this building is under review and is to be confirmed.* When not in use, laboratory books will be locked away by Data Users and there will be restricted access to the key.

b) Security of electronic data
As far as possible, patient identifiable data will be stored on the University of Leeds network file storage. Occasionally such data will be stored on laptop hard drives and an encrypted password-protected space will be used. In the short term, it is possible that the Data Users Dr Rosalyn Jewell and Mrs Sandra Tovey may need to store confidential data on their laptop hard drives. These Data Users both use Macs and so the Disk Utility software outlined already (5. COMPUTER SOFTWARE) will be used to encrypt the Mac hard drives.

Any email attachments containing personal data will be encrypted. This includes emails from the Trials' Offices to the clinicians in the local clinical centres and Data Users, and emails from the Data Users to external collaborators. These email attachments will be deleted from sent boxes and inboxes when no longer required.

It is not envisaged that the Data Users will need to copy patient data onto removable media, e.g. memory sticks or discs, for the Interferon Study. The Section of Epidemiology and Biostatistics rarely uses removable media to transfer data. Our current policy is that such media must be encrypted (see 5. COMPUTER SOFTWARE).

fmpro1, fmpro2, xserve1 and CRUK 2003 servers
The servers fmpro1, fmpro2, xserve1 and CRUK 2003 are all located in one server room in a University building at SJUH. The room is protected by a lock with access to the key restricted to nominated staff. Out of hours access to the building is controlled by swipe card. The servers operate a level of RAID functionality which protect against single drive failure.

The servers are protected by a local firewall and the University's firewall. The University of Leeds campus border firewall blocks all network traffic from the internet to the servers.

bcos and bcos2 servers
The servers bcos and bcos2 are in a network hub room in a University building at SJUH. This room is locked and access to the floor containing the room is controlled by swipe card. The servers each have their own as well as the University's firewall.

c) General security
The Data Users will be based in several rooms in the Cancer Genetics building. The Section of Epidemiology and Biostatistics is the sole occupant of the first floor of the two-storey Cancer Genetics building. Access is via steps to the first floor. There is no public access, but there is a meeting room, seminar room and coffee area that is available for use by other members of the Cancer Research UK Clinical Centre (of which the Section is also a member).

Access to the building is through one of three doors which are kept locked and access granted by electronic keycard authorization. All members of the Clinical Centre based on the SJUH campus have a keycard. Every door and window in the building is kept locked when no member of the Section is present. All room doors have mortis locks**.**

The building has a burglar alarm that is activated using a keypad using a code that is available to all members of the Section. SJUH security personnel are alerted if the alarm goes off. Each member of the Section is responsible for any external personal that they invite into the building.

If an office room is to be empty for some time then it must be locked, with the windows closed. All desktop computers are locked in areas where there is a greater chance of visitor or non-Section staff having access, and locks are provided for all laptops or laptops are locked away when not in use with restricted access to the key.

A clear desk policy will be followed to ensure that if a Data User is working on confidential data then that data must be secured prior to the Data User leaving their workstation.

The Data Users who work in the laboratory use laboratory facilities on the first floor of the Cancer Medicine Research Unit building. This building is run by the University, NHS and Cancer Research UK. During working hours access to the building is provided by a front and back door both secured via keypad authorisation. Visitors can only access the building via the front door where they are signed in at the reception and can only access further if accompanied by an appropriate member of staff. During non-working hours access to the building is via the front door only, the rear door being secured by a mortice lock.

10. ACCESS CONTROL

Access to the University of Leeds network is controlled by the University's Information Security Policy. Access to Interferon Study data will be additionally subject to this security policy.

a) fmpro1, fmpro2, xserve1 and CRUK 2003 servers
Access to the servers is controlled locally by the Server Managers using an Apple Macintosh Open Directory Server or local server accounts through login IDs and 'strong' passwords. Access to servers from off-campus via secure encrypted links (SSL) is permitted by authorized users – this access is in the process of being changed to only allow access via a new server cluster's built-in VPN server (http://www.apple.com/server/macosx/features/networking.html). Access to patient identifiable data from privately owned machines is not allowed.

Access to each database (the FMPro databases and Access DNA database) is restricted by username and password or password only. Access to databases and electronic files containing patient identifiable data is authorized by the Information Custodian; if the Information Custodian is notcontactable and urgent access is required then the Project Manager may authorize access, but must inform the Information Custodian of this. Authorised access is then actioned by the server manager, at the level of the server, and the data manager, at the level of the database.

For Data Users, desktop passwords must be at least eight characters long and of a complex type (letters, numbers and characters). Passwords must not be disclosed to any unauthorised user of the network and only disclosed to senior members of staff for reasons at the discretion of the Section leader. Passwords should be changed at least once every six months.

Every Data User should be fully aware of password security and every user has the following responsibilities:

- must maintain security of their password;
- must use passwords that are not easily deciphered;
- must not disclose their passwords to anyone else;
- passwords must be different from their account name;
- passwords should not be the account holder's name;
- words commonly found in the dictionary should not be used;
- passwords must never be written down.

In addition, if a new Data User joins the server they must change their password within the first five days after their account has been made active.

Whenever a Data User leaves the room for a substantial amount of time they should either log-out of the system or use a password-protected screen saver; personal data should never be left displayed on the screen.

b) bcos and bcos2 servers

bcos2 has an internal 192.168.1. connection that is accessible locally only. Only ssh (from limited locations) and https is allowed from bcos.

Access to bcos and bcos2 is via a password protected web interface. Each Data User has a separate username and password. Read and write access to each data set on the servers is controlled by the Data User who created the data set. The superuser of these servers is Dr Jenny Barrett who is also a Data User.

11. ACCESS CONTROL MATRIX

Table 5 lists Data Users who have access to electronic personal data and Table 6 lists Data Users with access to paper personal data.

Table 5: Access control matrix for electronic personal data

Electronic data name	**Name of server hosting electronic data**	**Users with access to area on server containing electronic data**	**Data Users with access to electronic data**
FMPro Histopathology database	fmpro1 or fmpro2*	Sandra Tovey, May Chan and *Data Users to be confirmed as database in development*	Sandra Tovey, May Chan and *other Data Users to be confirmed as database in development*
FMPro Interferon Study database	fmpro1 or fmpro2*	Rosalyn Jewell, May Chan, Catherine Plant and Julia Newton-Bishop	Rosalyn Jewell, May Chan, Catherine Plant and Julia Newton-Bishop
Excel DNA extraction and RNA extraction spreadsheets Excel list of samples for laboratory tests	xserve1	Lab 1 users: Tim Bishop, Julia Newton-Bishop, Juliette Randerson-Moor, Kairen Kukalizch, Caroline Conway**, Mark Harland, Samira Lobu, Rosalyn Jewell and laboratory technician (to be appointed)	Lab 1 users: Tim Bishop, Julia Newton-Bishop, Juliette Randerson-Moor, Kairen Kukalizch, Caroline Conway**, Mark Harland, Samira Lobu, Rosalyn Jewell and laboratory technician (to be appointed)
Excel, Stata and SAS data sets created from laboratory test results and/or clinical data	xserve1	Rosalyn Jewell, Faye Elliott, Ruby Chang, Jenny Barrett, Julia Newton-Bishop and May Chan	Rosalyn Jewell, Faye Elliott, Ruby Chang, Jenny Barrett, Julia Newton-Bishop and May Chan
Access DNA database	CRUK 2003	Lab 1 users: Tim Bishop, Julia Newton-Bishop, Juliette Randerson-Moor, Kairen Kukalizch, Caroline Conway**, Mark Harland, Samira Lobu, Rosalyn Jewell and laboratory technician (to be appointed)	Juliette Randerson-Moor and Kairen Kukalizch
BC gene database	bcos and bcos2	Jenny Barrett, John Taylor***, Jeremie Nsengimana***, Mark Iles***, Rosa Parisi***, Tim Bishop and Rosalyn Jewell	Rosalyn Jewell, Jenny Barrett and *other Data Users to be confirmed*

* Depending on whether remote access is required as only fmpro2 will allow this
** Not a Data User, but a member of the Section's laboratory group
*** Not a Data User, but a member of the Section's statistical group

Table 6: Data Users with access to paper personal data.

Paper data name	Data User who has access
Histopathology report	Sandra Tovey
List of samples to be used in genetic tests	Rosalyn Jewell, Tim Bishop, Julia Newton-Bishop, Juliette Randerson-Moor, Kairen Kukalizch, Caroline Conway*, Mark Harland, Samira Lobu and laboratory technician (to be appointed)
Lab book	Rosalyn Jewell, Tim Bishop, Julia Newton-Bishop, Juliette Randerson-Moor, Kairen Kukalizch, Caroline Conway*, Mark Harland, Samira Lobo and laboratory technician (to be appointed)

* Not a Data User, but a member of the Section's laboratory group

12. AUDIT TRAIL

See section 6. Quality Audit in the document 'Standard Operating Procedure (SOP) for the Management of Tumour Blocks'. Furthermore, there will be an email trail of clinical data sent to the Data Users by the clinical trail offices.

13. DATA QUALITY & RETENTION

Any confidential paper data will be shredded using one of the Section's cross cutting shredders and then will be disposed of using the facilities provided by the Leeds Teaching Hospital Trust (http://www.leedsth.nhs.uk/foi/policies_and_procedures.php?systemID=19&fileID=141).

Once clinical data have been transferred to the FMPro Interferon Study database, the encrypted email attachment containing these data will be deleted.

If server hard drives from the fmpro1, fmpro2, xserve1 and CRUK 2003 servers are ever removed from a system they are stored offsite and marked with date of removal and date when they can be recycled. If these drives are ever reused or recycled then they are given a 7-pass clean erase and marked that they used to be server drives. If a system that the drives are in is set for disposal the drives are either retained for reuse or physically destroyed. The server drives are never passed out of the control of the server managers.

If server hard drivers from the bcos and bcos2 servers fail then they will be kept for spare parts and when there are no useful parts left they will be physically destroyed. These servers have a RAID 5 file system and if disks were ever removed for reuse then they will be erased and will not leave the building wherethey are currently located.

When data are stored on encrypted laptop drives, it will be securely and permanently removed using agreed software to recognized standards: the data deletion method is built in to the Operating System and 7-pass clean erase is normally used which meets the US Department of Defence (DOD) 5220-22 M standard for securely erasing magnetic data. This method applies to both Mac and PC hard drives.

Following the designated period of 20 years, if the data are no longer required, it will be deleted from the server which will result in the removal of the data from the server backup tapes; this applies to data from fmpro1, fmpro2, xserve1 and CRUK 2003 servers.

14. DATA BACKUP

a) fmpro1, fmpro2, xserve1 and CRUK 2003 servers
The servers are subject to a daily full backup to the backup files server which in turn archives to tape media daily. The backup regime currently employed involves a daily incremental backup and weekly full backup to tape media. Tapes are rotated on an eight week cycle. A tape containing a full backup is removed each week and kept off-site for six months. Tapes are held in a secure location on the University campus, at a different site to the servers.

Backup data are not encrypted currently but should be by the end of August 2008. NetVault's built-in CAST-128 Encryption Algorithm (a 128-bit ECB encryption mode) will be used.

Data Users are responsible for ensuring data held temporarily on local hard drives are backed up to network file storage facilities.

b) bcos and bcos2 servers
Backups are taken from bcos to disks on bcos2 and to a machine in another University building at SJUH. Backups are taken weekly and are retained for up to five weeks. Approximately every two months a backup is placed on an external hard drive.

15. SECURITY INCIDENT MANAGEMENT & REPORTING

The Data Manager, System Managers, Server Managers and Data Users will be responsible for reporting any incidents which may lead to potential breaches of security. Incidents will be reported to the Information Custodian who will report them to the Section leader, who is responsible for the overall management of the SLSP, and to the Chief Investigator of the Interferon Study. Any serious failures to comply with PIAG conditions of approval for the Interferon Study will be reported to the PIAG Secretariat by the Information Custodian.

16. BUSINESS CONTINGENCY PLAN

In the event of any failure of data collection, storage or data retrieval system, the Data Manager and System Managers will be responsible for identifying:

- The cause of the failure;
- The action necessary to resolve the failure (see 17. Disaster Recovery);
- The anticipated time taken to resolve the failure;
- The consequence of the failure;
- Contingency actions to maintain the Interferon Study (see 17. Disaster Recovery).

It is envisaged that if there is a failure of any part of the project cycle, preceding stages of the project will be postponed until this has been resolved or a contingency solution has been implemented (see 17. Disaster Recovery). Hence, if it is not possible to retrieve data, further collection and storage will be postponed until this is resolved.

17. DISASTER RECOVERY

Table 7 lists disasters that may occur during the Interferon Study and the action that will be taken to recover from them.

Table 7: Disaster Recovery

Incident	Action
Loss of key personnel	Second System Manager will cover lost System Manager Data Manager will be replaced on instruction of Information Custodian
Loss of laptop/desktop	Computer will be replaced by another computer. Confidential data are stored in encrypted spaces on laptop hard drives (see 5. Computer Software) and are not stored on desktop hard drives
Loss of network	Data Manager will relocate to part of campus with functioning network. If the whole University network is not working, and recovery is expected to be greater than three days, the system will be downloaded from the server onto the computer and used in stand-alone mode.
Loss of server	Files will be recovered from tape and installed on stand alone computer. The System Managers will have overall responsibility for managing this process.
Loss of data	Trial clinical data will be requested from trials' offices again University of Leeds server data will be recovered from backup

18. CHANGE CONTROL

This project is anticipated to run until completion with a well defined scope and outputs. No major changes are expected but any changes that are required will be reported to the Information Custodian for authorization before implementation.

Dr Catherine Plant
Project Manager
Section of Epidemiology and Biostatistics
30th July 2008

22.4 Information leaflet for patients

You previously took part in a clinical trial regarding melanoma. We are carrying out further research into melanoma and would like to inform you of this research, and give you the opportunity to contact us if you do not want us to use your samples or data for the study.

If you decide not to take part your clinical care will not be affected. You can decide to opt out of this study at any time.

Aims of our study
Within each cell in our body there are packets of information called genes which influence everything about us, including characteristics such as eye colour and how tall we are. We already know that there are genes which can increase the risk of certain cancers, including melanoma, and genes that influence how we respond to treatments, for example interferon.

Our aim is to identify genetic differences that will help us identify patients who will obtain a benefit from interferon treatment and those who will develop side effects. In the long-term this will help us choose the best treatment options for each patient individually. This research has only recently become possible because of scientific advances. It was not possible when you took part in the interferon clinical trial.

Why is this relevant to you?
You have previously been involved in a study in which you were either given interferon or no further treatment, after your operation to remove your melanoma and maybe also local lymph nodes. In this study we are using stored samples of your melanoma, any skin samples and any lymph nodes that were removed. Tissue samples like this are routinely stored in the hospital pathology laboratory for many years in case they are needed. From these samples we will identify genetic changes.

What will happen to my tissue samples?
With the help of the team who originally recruited you to the interferon trial, we will find your tissue samples that were removed, and transfer them to Leeds for analysis.

At your local hospital, the samples will be labelled in such a way that the laboratory researchers working on your sample will never have the name of the person the samples have come from.

From the sample we will extract genetic material and analyse the genes inherited from your parents and genetic changes in the melanoma cells. We will then identify any changes and see if they are linked to whether you have suffered a relapse or not, or if you received benefit or developed side effects from interferon treatment or not.

When the study ends remaining tissue will be returned to your local hospital. We will keep the small pieces of tissue and the genetic material we have removed. These samples will be kept for up to 20 years and may be used for ethically approved melanoma research in the future. These samples may be transferred to other researchers across the world, however they will be labelled with study number only and no other information identifying the samples as being removed from you.

What information do you need and what will happen to my information?
We will need information about your original melanoma, whether you have suffered a relapse and how you responded to your interferon treatment if you received it. This information has already been obtained for the purposes of the original interferon trial, so this information will be sent to us in Leeds. Again, this information will be labelled in such a way so researchers will not be able to tell whom the information relates to. We plan to keep this information for 20 years and it may be used again for the purposes of ethically approved research. This may involve transfer to other researchers in the world and in this case your information will be labelled by study number only.

What do you need to do?
The information we require has already been obtained for the purposes of the original clinical trial and the samples are in your local hospital pathology departments so we do not need any further information or samples from yourself, and we will not need to contact you in the future.

Are there any risks involved?
The information we obtain about you and your melanoma will be kept strictly confidential as will any genetic information we obtain during the study.

What are the benefits for you?
Involvement in this study will not give you any benefits in the short term. In the long term, this study may help us identify genetic changes that will help predict benefit from interferon treatment. This may be beneficial to you in the future if you need further treatment and will be essential to patients who are diagnosed with melanoma in the future. However, overall the research is unlikely to benefit you directly.

Will you be informed of any results?
We are unable to provide individualized results from this study. This is because the true meaning of these results is unlikely to be clear for a long time. However, we expect to make the general study findings available as a plain English summary on the GenoMEL website (www.genomel.org).

This information sheet and the study protocol are available on the GenoMEL website (www.genomel.org).

If you do NOT want to be involved or have further questions

If you would not like us to use you clinical information or tissue samples, you will need to inform us.

Please contact:

Dr. Rosalyn Jewell
Section of Epidemiology and Biostatistics,
Leeds Institute of Molecular Medicine,
University of Leeds,
Cancer Genetics Building,
St. James's University Hospital,
Beckett Street,
Leeds,
LS9 7TF
E-mail: R.A.Jewell@leeds.ac.uk
Phone: 01132065037
01132064573

Please take time to read this information carefully and discuss it with your friends, relatives and GP if you wish.

Leeds Institute of Molecular Medicine

The Leeds Institute of Molecular Medicine (LIMM) is part of the University of Leeds and dedicated to research into defining the molecules involved in human diseases and in research to convert these studies into therapies. This research will take place in the Section of Epidemiology and Biostatistics based at St. James's Hospital in Leeds. The focus of this group is on the contribution of genes to the incidence of disease in the general population and the joint effects of genes and environment. Particular research interests of this group involve the study of melanoma.

CANCER RESEARCH UK

Cancer research UK is the UK's leading charity dedicated to cancer research. Our research group receives funding from this charity to continue cancer related research.

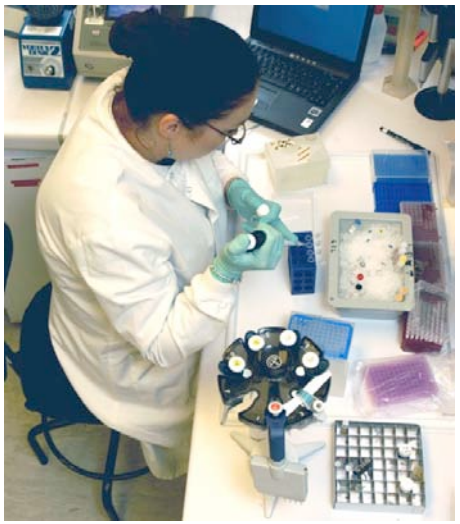
The Leeds Teaching Hospitals NHS
NHS Trust

Leeds Teaching Hospitals NHS Trust is the largest trust in the UK and St. James's Hospital is the largest teaching hospital in Europe. St. James's is the regional centre for cancer care and this research will take place on the St. James's Hospital site in the Leeds Institute of Molecular Medicine.

UNIVERSITY OF LEEDS

Study Information Leaflet

Predicting Benefit From Interferon: Personalised Therapy for Melanoma



This leaflet is for people who took part in interferon trials (EORTC 18952, and 18991, Aim High)

Version 1
29/07/08

22.5 Letters of Collaboration

Karolinska Institutet

Date
7/2/2008

Department of Oncology-Pathology
CancerCentre Karolinska
Johan Hansson,
Head of Melanoma Unit
johan.hansson@ki.se

Professor Julia A Newton Bishop
Section of Epidemiology and Biostatistics,
Leeds Institute of Molecular Medicine,
University of Leeds,
Cancer Genetics Building,
St James's University Hospital,
Beckett Street,
Leeds LS9 7TF
United Kingdom

Letter of collaboration

I hereby confirm on behalf of the Nordic Melanoma Collaborative Group that we will participate in the study "Predicting Benefit from Interferon Therapy: Personalized Therapy for Melanoma" coordinated by Professor Julia A Newton Bishop, University of Leeds.

Johan Hansson, MD, PhD
Associate Professor, Consultant

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EORTC
European Organisation for Research and Treatment of Cancer
AISBL International Non-Profit Association under Belgian law IVZW

Pr. Alexander Eggermont
Dep.: Dept of Surgical Oncology
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Fax: +31 10 7041011 / 1034
a.m.m.eggermont@erasmusmc.nl

Dear Julia,

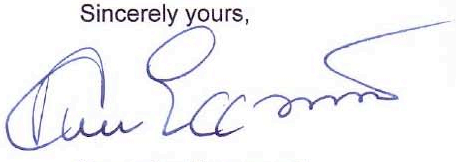
I am very happy to agree to a collaboration between the EORTC Melanoma Group, CHEMORES and the University of Leeds.

This collaboration will allow us to perform a study that no single group could perform alone. Moreover it will benefit from established collaborations within EORTC and the FR6 funded CHEMORES integrated project.

The questions asked are key to the aims of CHEMORES and the collection of RNA and DNA as described in the grant application are funded by the CHEMORES grant.

The project is novel and important in terms of the questions asked and as proof of principal that large scale collaborative projects of this type will provide answers to the issues of identifying biomarkers.

Sincerely yours,



Alexander Eggermont

The University Of Sheffield.

School Of Medicine & Biomedical Sciences.

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Academic Unit of Clinical Oncology
Professor B W Hancock
YCR Director of Cancer Research
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BWH/JP

23rd June 2008

Professor J A Newton Bishop
Professor of Dermatology
Section of Epidemiology & Biostatistics
Leeds Institute of Molecular Medicine
University of Leeds
Cancer Genetics Building
St James's University Hospital
Beckett Street
LEEDS
LS9 7TF

Dear Professor Newton Bishop

Re: Predicting benefit from interferon adjuvant therapy for melanoma: personalised therapy for melanoma

I am pleased to confirm my agreement to collaborate in this study.

Yours sincerely

Professor B W Hancock
YCR Director of Cancer Research

In partnership with:
Sheffield Teaching Hospitals NHS Foundation Trust
Weston Park Hospital Cancer Appeal

The Queen's Anniversary Prizes
1998 2000 2002

University of Pittsburgh

University of Pittsburgh Cancer Institute

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July 1, 2008

Julia A Newton Bishop
Professor of Dermatology,
Section of Epidemiology and Biostatistics
Leeds Institute of Molecular Medicine
University of Leeds
Cancer Genetics Building
St James's University Hospital
Beckett Street
Leeds LS9 7TF

Re "Predicting benefit from interferon treatment: personalized therapy for melanoma"

Dear Julia,

We would be happy to collaborate on the above project with you. As you know, we have investigated the role of interferon in melanoma patients for many years. We have a major interest in understanding the mode of action of this drug, and have treated many patients in collaborative trials over the years.

If your group does identify biomarkers of benefit and or toxicity from interferon, we would be extremely interested to validate your findings in our samples.

Sincerely,

John M. Kirkwood, MD
Usher Professor of Medicine and Dermatology
Vice Chairman/Clinical Research, Dept. of Medicine
Director, Melanoma and Skin Cancer Program
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NCI CCC A Comprehensive Cancer Center designated by the National Cancer Institute