ISTITUTO PER LO STUDIO
E LA PREVENZIONE ONCOLOGICA

DELIBERAZIONE DEL DIRETTORE GENERALE
(Nominato con D.P.G.R.T. n. 233 del 13/12/2010)

N° 111 del 27/09/2012

Oggetto: Progetto "Identification and clinical evaluation of genetic and epigenetic multiple markers in cell-free plasma DNA of melanoma patients" finanziato dall'Università degli Studi di Firenze, Dipartimento di Fisiopatologia Clinica. Approvazione del progetto, recepimento del finanziamento ed approvazione del piano economico finanziario.	
Struttura Proponente	S.C. Epidemiologia ambientale occupazionale
	Contabilità e Controllo di Gestione
	Coordinatore Amministrativo
Proposta n. 111	Responsabile del procedimento
	Estensore Marco Di Maio

IMMEDIATAMENTE ESEGUIBILE [X]

Conto Economico n. 3A01020301

Eseguibile a norma di Legge dal 27 SET. 2012

Pubblicato a norma di Legge il 28 SET. 2012

Inviato al Collegio Sindacale il 28 SET. 2012

L'anno 2012, il giorno 27 del mese di Settembre
Il sottoscritto prof. Gianni Amunni, nella sua qualità di

DIRETTORE GENERALE

di questo Istituto per lo Studio e la Prevenzione Oncologica , con sede in Via Cosimo Il Vecchio 2 – 50139 Firenze, in forza del Decreto del Presidente della Giunta Regionale Toscana n. 233 del 13/12/2010.

Visto il D. Lgs.vo 30/12/1992 n. 502 e sue successive modifiche ed integrazioni e la L. R. Toscana n. 40 del 24/02/2005 di disciplina del Servizio Sanitario Regionale e successive modificazioni ed integrazioni;

vista la legge regionale 4 febbraio 2008, n. 3, così come modificata dalla Legge R.T. n. 32/12, ai sensi della quale è stato istituito l'ISPO – Istituto per lo Studio e la Prevenzione Oncologica - "ente del servizio sanitario regionale, dotato di personalità giuridica pubblica e di autonomia organizzativa, amministrativa e contabile" (art.1);

vista la delibera del Direttore Generale n. 4 del 12.01.2012 con la quale è stato approvato il regolamento dei progetti finalizzati;

premesso che:

- la Regione Toscana con Decreto Dirigenziale n. 6368 del 30.12.2008 avente ad oggetto "Istituto Toscano Tumori – approvazione bando e relativa modulistica per la presentazione di progetti di ricerca in campo oncologico – anno 2008" ha approvato e di seguito diffuso un avviso pubblico;
- la Regione Toscana in attuazione del suddetto Bando, con Decreto Dirigenziale n. 7197 del 29.12.2009 ha approvato il progetto della durata di tre anni, denominato"Identification and clinical evaluation of genetic and epigenetic multiple markers in cell-free plasma DNA of melanoma patients" presentato dall'Università degli Studi di Firenze, Dipartimento di Fisiopatologia Clinica, Responsabile del Progetto Prof. Claudio Orlando;
- il suddetto progetto è stato approvato con atto convenzionale tra la Regione Toscana e l'Università degli Studi di Firenze, Dipartimento di Fisiopatologia Clinica con durata dal 15.04.2010 al 14.04.2013;
- nell'atto convenzionale sopra citato era prevista la partecipazione di ISPO alla realizzazione del progetto come additional unit per il secondo ed il terzo anno;

vista la nota prot. 82, class III/13.3 del 24.01.12, dell'Università degli Studi di Firenze, unita alla presente sotto lettera "A" quale parte integrante e sostanziale, con la quale si comunica l'impegno da parte del Dipartimento di Fisiopatologia Clinica dell'Università di Firenze a trasferire ad ISPO, secondo le modalità stabilite dalla convenzione tra Regione Toscana e l'Università degli Studi di Firenze - Dipartimento di Fisiopatologia Clinica (allegato alla nota sopra citata), la somma complessiva di Euro 33.000,00 (trentatremila/00) per la realizzazione da parte di ISPO, in qualità di additional unit, del progetto "Identification and clinical evaluation of genetic and epigenetic multiple markers in cell-free plasma DNA of melanoma patients";

rilevato che ISPO, avendo ricevuto la nota sopra citata in data 6.3.2012, è stato coinvolto nel progetto solo per il terzo anno;

viste la rendicontazione e la richiesta di rimodulazione, presentate all'ITT da parte dell'Università degli Studi di Firenze – Dipartimento di Fisiopatologia Clinica alla scadenza del secondo anno del progetto, agli atti, dalle quali si evince che l'Additional Unit ISPO non ha effettuato spese nel secondo anno del progetto e, conseguentemente, si richiede all'ITT di poter utilizzare i fondi residui del secondo anno nel terzo anche per la Additional Unit ISPO;

vista la nota della Regione Toscana, Giunta Regionale prot. n AOOGRT/186221/Q.80.110 del 28/6/12 con la quale si approva il rendiconto economico e la richiesta di rimodulazione del piano economico finanziario da parte dell'Università di Firenze;

rilevato che il Responsabile del Progetto per ISPO, Dr.ssa Lucia Miligi, Biologo Dirigente la S.C. di Epidemiologia ambientale occupazionale, ha presentato una relazione progettuale per la realizzazione degli obiettivi previsti dal progetto, unita alla presente sotto lettera "B" quale parte integrante e sostanziale;

ritenuto pertanto opportuno di recepire il finanziamento pari a € 33.000,00 (trentatremila/00) ed il relativo piano economico finanziario, unito alla presente sotto lettera "C" quale parte integrante e sostanziale, dando atto che il previsto ricorso a collaborazioni professionali esterne è condizione fissata dall'Ente finanziatore, il quale ha espressamente escluso la possibilità di utilizzo del personale dipendente nelle attività progettuali;

ritenuto di dichiarare il presente atto immediatamente eseguibile per consentire in tempi brevi l'avvio del progetto;

vista la delibera del Direttore Generale n. 5 del 14.07.2008 con la quale è stato approvato il regolamento dell'ISPO;

con il visto di conformità giuridico amministrativa del Coordinatore Amministrativo;

con il parere favorevole del Direttore Sanitario

DELIBERA

Per quanto esposto in narrativa, formante parte integrante e sostanziale del presente atto:

1. di recepire il finanziamento derivante dal progetto "Identification and clinical evaluation of genetic and epigenetic multiple markers in cell-free plasma DNA of melanoma patients" tramite l'Università degli Studi di Firenze, Dipartimento di Fisiopatologia Clinica come da allegato "A" quale parte integrante e sostanziale;

2. di prendere atto della relazione progettuale e del relativo piano economico finanziario redatto in coerenza con il piano presentato nel Grant Proposal 2008 approvato da ITT, predisposti dalla dr.ssa Lucia Miligi, Responsabile del Progetto per ISPO, documenti allegati rispettivamente sotto le lettere "B" e "C" quali parti integranti e sostanziali;

3. di prendere atto che ISPO, per l'effettuazione delle attività connesse al progetto, riceverà dall'Università degli Studi di Firenze, Dipartimento di Fisiopatologia Clinica la somma di Euro 33.000,00 (trentatremila/00), a valere dei ricavi registrati nel bilancio d'esercizio dell'Istituto per il periodo di competenza relativo, aut. N. 63/12, cdc 675, conto economico 3A01020301 "contributi da altri sanitari";

4. di dichiarare il presente atto immediatamente eseguibile ai sensi della normativa vigente;

5. di trasmettere il presente atto all'albo di pubblicità degli atti di questo Istituto e al Collegio Sindacale.

IL DIRETTORE GENERALE
Gianni Amunni

IL DIRETTORE SANITARIO
Chiara Neri

Elenco degli allegati

Allegato A	Nota del Dipartimento di Fisiopatologia Clinica – Università di Firenze e allegati	pagg. 38
Allegato B	relazione progettuale del Responsabile del progetto	pag. 01
Allegato C	piano economico finanziario	pag. 01

Strutture aziendali da partecipare:

S.C. Epidemiologia ambientale occupazionale ISPO;
S.S. Contabilità e Controllo di Gestione ISPO;
Gestione Contabile Progetti ISPO;
Supporto Amministrativo Attività Scientifica e di Ricerca ISPO;
Dipartimento Amministrazione e Finanza ASF.

ALLEGATO "A" ALLA DELIBERA D.G. N. 111 DEL 19/09/2012

Università degli Studi di Firenze
DIPARTIMENTO DI FISIOPATOLOGIA CLINICA

Prot 82 class III/13.3
del 26/1/2012

ISPO
Ufficio Supporto Amministrativo Attività Scientifica e di Ricerca
c. a. Monica Di Stasio
Via Cosimo il Vecchio 2
50139 Firenze

Oggetto: **ITT Grant 2008 - "Identification and Clinical Evaluation of Genetic and Epigenetic Multiple Markers in Cell-Free Plasma DNA of Melanoma Patients", responsabile scientifico Prof. Claudio Orlando**

Con la presente si comunica l'impegno da parte del Dipartimento di Fisiopatologia Clinica a trasferire complessivamente € 33.000,00 a favore dell'I.S.P.O., ai sensi della convenzione tra Università degli Studi di Firenze, Dipartimento di Fisiopatologia Clinica e Regione Toscana per il progetto di ricerca in oggetto, che vede tra i partner codesto Istituto fin dalla stesura del progetto stesso.

Il contributo verrà trasferito dal Dipartimento per stati di avanzamento e conformemente ai modi e alla tempistica con cui la Regione Toscana trasferirà il finanziamento al Dipartimento.

Si allega copia della convenzione, della richiesta di finanziamento e del budget rimodulato.

Si trasmette inoltre un modello per la comunicazione del conto corrente dedicato ai sensi dell'art. 3 della Legge 136/2010, che vi preghiamo di restituirci, debitamente compilato e sottoscritto.

Distinti saluti

Il Direttore del Dipartimento
Prof. Stefano Milani

Prof. Gianni Amunni
Direttore Generale ISPO

ISTITUTO PER LO STUDIO E LA PREVENZIONE ONCOLOGICA
I.S.P.O.
06 FEB. 2012
Prot. N. 320
Pos.

All. 1

REGIONE TOSCANA

Giunta Regionale

Progetti di ricerca ITT

CONVENZIONE

tra la Regione Toscana e l'Università degli Studi di Firenze per la realizzazione del progetto "Identification and Clinical Evaluation of Genetic and Epigenetic Multiple Markers in Cell-Free Plasma DNA of Melanoma Patients"

L' anno 2010, il giorno ...15..... **del mese di** APRILE 2010

La **REGIONE TOSCANA**, (di seguito chiamata Regione), Codice Fiscale 01386030488, rappresentata dal dirigente regionale Dr. Valerio Del Ministro, nato a Pescia (PT) il 10/07/1950, domiciliato presso la sede dell'Ente via T. Alderotti 26/in Firenze, nominato con decreto del Direttore Generale n. 1530 del 05.04.2007, Responsabile del Settore "Assistenza sanitaria" della Direzione Generale del Diritto alla Salute e delle Politiche di Solidarietà, autorizzato, ai sensi della L.R. 44/2003, a sottoscrivere la presente convenzione approvata in schema con decreto n. 4845 del 13/10/2008;

E

L'Università degli Studi di Firenze, Codice fiscale 01279680480 in persona del Direttore del Dipartimento di Fisiopatologia Clinica, Prof. Stefano Milani, nato a Firenze il 17/05/1955;

PREMESSO

- che la Regione con Decreto Dirigenziale n. 6368 del 30.12.2008 avente per oggetto "Istituto Toscano Tumori - approvazione bando e relativa modulistica per la presentazione di progetti di ricerca in campo oncologico - anno 2008" ha approvato e di seguito diffuso un avviso pubblico;

- che la Regione in attuazione del suddetto Bando, con Decreto Dirigenziale n. 7197 del 29 Dicembre 2009 ha approvato il progetto, denominato "Identification and Clinical Evaluation of Genetic and Epigenetic Multiple Markers in Cell-Free Plasma DNA of Melanoma Patients",

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Settore Assistenza Sanitaria

DIPARTIMENTO DI FISIOPATOLOGIA CLINICA
UNIVERSITA' DEGLI STUDI DI FIRENZE

- che l'Università degli Studi di Firenze, è in possesso dei titoli e dei requisiti richiesti dal Bando in oggetto per svolgere correttamente il progetto di ricerca;

- che il responsabile del progetto di ricerca è il Dr. Claudio Orlando, nato a Firenze il 16 agosto 1952;

- che il responsabile del progetto di ricerca condivide il contenuto della presente convenzione;

TUTTO CIÒ PREMESSO SI CONVIENE E SI STIPULA QUANTO SEGUE:

Art. 1

Premessa

La premessa costituisce parte integrante ed essenziale della presente convenzione.

Art. 2

Soggetto attuatore

L'Università degli Studi di Firenze, successivamente indicato anche come "soggetto attuatore", si impegna verso la Regione, a curare l'organizzazione e l'effettuazione delle attività indicate nel progetto di ricerca come sopra denominato, successivamente indicato semplicemente progetto, di cui si allega copia sotto la lettera A), quale parte integrante ed essenziale della presente convenzione.

Il soggetto attuatore, per la realizzazione delle attività progettuali, dovrà avvalersi di personale in possesso delle necessarie caratteristiche professionali specifiche; e si impegna al pieno rispetto della normativa in vigore, in particolare quella relativa alla sicurezza sui luoghi di lavoro. La Regione resta comunque estranea a qualsiasi rapporto di lavoro e collaborazione a qualunque titolo instaurato dal soggetto attuatore nello svolgimento e per le necessità del programma.

Art. 3

Finanziamento del progetto

Il progetto è finanziato dalla Regione per un costo complessivo di €. 181.000 (centoottantunomila), secondo le modalità di erogazione previste al successivo art. 4, così ripartite, 1° anno: €. 61.000 (sessantunomila), 2° anno: €. 60.000 (sessantamila), 3° anno: €. 60.000 (sessantamila).

Il soggetto attuatore dichiara altresì che il progetto è co-finanziato per €. 30.000 (TRENTAMILA), a carico del soggetto attuatore o di altri soggetti,

OVVERO ~~che non esistono co-finanziamenti~~.

In particolare il soggetto attuatore dichiara di non cumulare il finanziamento approvato con altri ulteriori finanziamenti pubblici o privati già ottenuti per realizzare le stesse azioni e che non verranno chiesti in futuro altri finanziamenti pubblici per la realizzazione delle stesse azioni.

Il finanziamento previsto a carico della Regione si deve intendere al lordo di qualsiasi

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spesa e costituisce il tetto massimo di spesa rimborsabile. L'erogazione dei rimborsi successivi al primo acconto sarà subordinata all'obbligo di documentare le spese effettivamente sostenute, come meglio precisato anche nei successivi articoli della presente convenzione. La mancata presentazione, senza giustificato motivo della documentazione suddetta, entro i termini previsti dai successivi articoli, comporterà la decadenza del diritto al rimborso, nonché il recupero degli acconti già erogati, fatte salve eventuali altre azioni per la tutela degli interessi della Pubblica Amministrazione nelle sedi opportune.

Poiché trattasi di un contributo di ricerca, il finanziamento è fuori campo di applicazione IVA e non è oggetto alla ritenuta di acconto del 4% ai sensi dell'art. 28 del DPR 600/73.

Art. 4
Erogazione del Finanziamento

La Regione, in conformità del Bando Regionale in oggetto, si impegna ad erogare le somme di cui al precedente art. 3, al ricevimento della documentazione richiesta e con le seguenti modalità:

Il finanziamento relativo al primo anno verrà erogato, salvo eccezioni, in due rate:

- la prima, pari al 70% dell'importo finanziato per il primo anno, dopo la comunicazione di inizio progetto di ricerca, firmata dal responsabile del progetto, contenente l'indicazione puntuale della data di avvio, non antecedente alla data del decreto di approvazione dei progetti.

- la seconda, pari all'ulteriore 30%, alla fine del primo anno dalla data dichiarata di inizio, inviando i seguenti documenti:

- Relazione scientifica sullo stato di avanzamento del progetto;
- Rendiconto delle spese effettivamente sostenute nell'intero primo anno di ricerca, da predisporre con gli stessi criteri utilizzati per la definizione del preventivo (vedi tab. 16 allegata al progetto). Il rendiconto dovrà esse redatto in relazione alle spese effettivamente sostenute per ogni singola voce del progetto, sulla base dei costi ammissibili previsti dalla normativa vigente;
- Dichiarazione sostitutiva resa ai sensi del DPR 28 Dicembre 2000, n. 445, con la quale il responsabile del progetto di ricerca attesta la veridicità ed esattezza dei dati esposti e dei documenti prodotti.

Si intende quindi che il 30% dei finanziamenti annuali saranno anticipati dal soggetto attuatore.

Eventuali spostamenti di somme tra voci di spesa o da un anno all'altro, nei progetti pluriennali, dovranno essere preventivamente autorizzati dalla Regione Toscana.

Tutti i documenti dovranno essere trasmessi entro 30 giorni dalla conclusione del primo anno del progetto.

L'esame della documentazione richiesta dovrà essere effettuato dagli organismi competenti entro 60 giorni dalla ricezione, in particolare la relazione scientifica sarà

sottoposta a valutazione del Direttore Scientifico dell'ITT; entro tale termine dovrà essere inoltre data comunicazione al responsabile del progetto sull'esito della verifica effettuata.

Per i progetti pluriennali, gli importi relativi agli anni successivi al primo saranno trasferiti annualmente sempre in due rate:

- la prima, pari al 70% dell'importo finanziato, insieme al saldo dell'anno precedente;

- la seconda alla fine dell'anno, seguendo le stesse modalità del primo anno.

Entro 60 giorni dal termine del progetto, dovranno essere inviati:

- la relazione scientifica conclusiva stilata dal responsabile del progetto, corredata di eventuali pubblicazioni che dovranno citare il supporto della Regione Toscana -ITT,

- il rendiconto economico finale complessivo secondo quanto indicato al successivo art. 6.

L'ultima rata del finanziamento verrà erogata a saldo delle spese effettivamente sostenute nella durata dell'intero progetto, rispettando il tetto massimo di spesa rimborsabile.

La documentazione finale dovrà essere approvata dall'Ufficio di Direzione dell'ITT entro 60 giorni dal ricevimento.

Ritardi o mancanza nel sottoporre questa documentazione potranno avere effetti negativi su successive richieste di finanziamento.

La Regione attuerà delle verifiche a campione sui documenti giustificativi di spesa utilizzando anche le strutture regionali competenti in materia. Il soggetto attuatore si impegna a restituire alla Regione gli importi da questa ricevuti, ma non riconosciuti ammissibili dall'Amministrazione stessa in sede di verifica finale del progetto, relativamente all'attività svolta. Il soggetto attuatore si impegna a restituire tali importi nelle forme e tempi indicati dalla Regione, fatto salvo il proprio diritto a prendere visione e controllare il verbale di accertamento finale delle spese, redatto in sede di verifica finale. L'eventuale recupero dei finanziamenti indebitamente ricevuti dal beneficiario finale sarà incrementato dagli interessi calcolati in base alla normativa vigente.

Art. 5
Durata del Progetto

Il progetto ha la durata di tre anni. Il non avvio del progetto e la conseguente mancata comunicazione entro 30 (trenta) giorni dalla firma del presente atto comporterà la risoluzione della convenzione e non potrà essere rimborsata nessun tipo di spesa

Il progetto non potrà subire né modifiche né proroghe salvo in casi del tutto eccezionali per i quali saranno definite apposite procedure. Il soggetto attuatore ed il responsabile del progetto di ricerca invieranno motivata e ben documentata richiesta scritta alla Regione che, acquisito il parere del Direttore Scientifico dell'ITT, accorderà o meno tale richiesta. La concessione della eventuale proroga, che sarà subordinata alla riconosciuta sussistenza di ragioni di necessità e d'opportunità, non costituisce motivo di maggiorazione del corrispettivo accordato.

Qualora sia accertata la mancata o irregolare attuazione del programma la Regione potrà sospendere l'erogazione del finanziamento.

Art. 6
Rendiconto finale

Il soggetto attuatore si impegna a presentare alla Regione, entro 60 (sessanta) giorni dal termine delle attività, il rendiconto finale relativo alle spese effettivamente sostenute nella durata dell'intero progetto. Tale rendiconto verrà presentato utilizzando la tabella 16 e specificando all'interno di ogni macrovoce le singole voci di spesa, anno per anno, sulla base dei costi ammissibili previsti dalla normativa vigente.

Questo dovrà essere corredato dalla relazione illustrativa delle attività svolte e da una dichiarazione sostitutiva resa ai sensi del DPR 28 Dicembre 2000, n. 445, con la quale il responsabile del progetto di ricerca attesti la veridicità ed esattezza dei dati esposti e dei documenti prodotti.

Per quanto concerne l'I.V.A. e le altre imposte dirette o indirette che possono essere recuperate, rimborsate o compensate in qualsiasi modo e che pertanto non restano definitivamente a carico del soggetto attuatore, beneficiario finale del finanziamento, non possono essere ammesse a rimborso.

I documenti giustificativi di tutte le spese sostenute nel corso dell'attuazione del progetto, dovranno essere conservati secondo le norme di legge ed esibiti in originale, su richiesta della Regione, per le verifiche previste dall'art. 4.

Art. 7
Responsabile del progetto di ricerca

Se nel corso della durata del progetto termina il rapporto di lavoro tra il responsabile del progetto ed il soggetto attuatore, il finanziamento si intende sospeso.
Se ne esistono i presupposti, il soggetto attuatore o il responsabile del progetto possono far richiesta alla Regione Toscana affinché il finanziamento venga corrisposto ad altro soggetto attuatore o ad altro responsabile.

Art. 8
Parere Comitato Etico

Se il progetto prevede la sperimentazione umana, l'utilizzo di campioni biologici umani o la sperimentazione su animali o loro campioni biologici, è richiesto il parere positivo del Comitato Etico locale prima dell'avvio della ricerca. Per quanto riguarda la sperimentazione umana e l'utilizzo di campioni biologici umani deve essere prestata particolare attenzione alla sussistenza dei requisiti in materia di consenso informato.

Art. 9
Proprietà dei risultati e pubblicazione

La proprietà dei risultati eventualmente brevettabili sarà definita in ottemperanza a quanto stabilito dal "Codice della Proprietà Industriale" emanato con D. Lgs. 10 febbraio 2005, n. 30 a norma dell'art. 15 della L. 12.12.2002, n. 273 salvo particolari accordi che

potranno essere stipulati anche successivamente tra le parti firmatarie del presente atto.

I proprietari dei risultati concedono l'uso degli studi, dei prodotti e delle metodologie sopra descritti per le finalità che le sono proprie, ferma restando la loro disponibilità in favore del Servizio Sanitario Regionale.

In caso di pubblicazione anche parziale dei risultati, è obbligatorio citare che la ricerca è stata fatta con il contributo della Regione Toscana-ITT.

Art. 10
Foro Competente

Per ogni controversia che dovesse insorgere con riferimento alla presente convenzione è competente il Foro di Firenze.

Art. 11
Trattamento dati personali

Il trattamento dei dati personali viene effettuato ai sensi dell'art. 18 comma 2 del Decreto Legislativo n. 196 del 30 giugno 2003, per l'esclusivo svolgimento delle funzioni istituzionali dell'ente.

Art.12
Oneri Fiscali

La presente convenzione è redatta in due originali. In caso d'uso verrà registrata a tassa fissa, ai sensi del II comma dell'art. 5 (caso d'uso) e dell'art. 38 (tassa fissa) del DPR 26.10.72, n. 634 e successive modifiche ed integrazioni, a cura e spese del richiedente la registrazione.

E' inoltre esente da bollo, ai sensi dell'art. 16, tab. B del DPR 26.10.72, n. 642, come modificato dall'art. 28 del DPR 30.12.82, n. 955.

Letto, approvato e sottoscritto

Per Regione Toscana (nome, cognome)

Per Ente/Azienda (nome, cognome)

DIREZIONE GENERALE DIRITTO ALLA SALUTE E POLITICHE DI SOLIDARIETA' Settore Assistenza Sanitaria

UNIVERSITA' DEGLI STUDI DI FIRENZE
DIPARTIMENTO DI FISIOPATOLOGIA CLINICA
Il Direttore
Prof. Stefano Milani

UNIVERSITA' DEGLI STUDI DI FIRENZE · DIPARTIMENTO DI FISIOPATOLOGIA CLINICA

All. 2

ALLEGATO B

ISTITUTO TOSCANO TUMORI (ITT) – REGIONE TOSCANA
GRANT PROPOSAL 2008

1. PRINCIPAL INVESTIGATOR (PI)

FIRST AND LAST NAME	CLAUDIO ORLANDO
POSITION	ASSOCIATE PROFESSOR
INSTITUTION	DEPARTMENT OF CLINICAL PHYSIOPATHOLOGY
ADDRESS	VIALE PIERACCINI 6
CITY	FLORENCE
PHONE	+39.0554271440
E-MAIL	c.orlando@dfc.unifi.it
LEGAL REPRESENTATIVE	PROF. AUGUSTO MARINELLI, RECTOR OF THE UNIVERSITY OF FLORENCE
ADDRESS	PZZA SAN MARCO 4
E-MAIL	rettore@unifi.it

2. PROJECT TITLE (Max 150 Characters)

Identification and Clinical Evaluation of Genetic and Epigenetic Multiple Markers in Cell-Free Plasma DNA of Melanoma Patients

2.1 KEY-WORDS

Cutaneous Melanoma	**Oncogene mutations**
Plasma Cell-Free DNA	**Prognostic markers**
DNA methylation	

3. ESTIMATED COMPREHENSIVE COSTS OF THE PROPOSED RESEARCH

Grant Requested to ITT	**375000 Euros**
Available Grant(s) co-finacing the Proposal	**60000 Euros**

4. PROJECT TIME-FRAME

Annual	
Biennal	
Triennal	**XXX**

5. COLLABORATORS INVOLVED IN THE PROJECT

FIRST AND LAST NAME OF THE COLLABORATOR	INSTITUTION (FULL NAME AND ADDRESS)
PROF DANIELA MASSI	**Department of Human Pathology and Oncology, viale Morgagni 85, 50134, Florence, Italy**
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Signatures

Principal Investigator	Legal Representative
Prof Claudio ORLANDO	

Date 19.2.2009

6. ABSTRACT (Max 2500 Characters) 2311 car.

DS-DNA fragments frequently occur in considerable amount in plasma of cancer patients.
Our proposal is based on the definition of a multiple markers panel of molecular alterations detectable in plasma cell-free DNA (CFDNA) in patients with cutaneous melanoma. The proposed panel includes the quantification of total CFDNA and its subsequent characterization for genetic and epigenetic alterations, including mutations of BRAF, NRAS and c-KIT oncogenes, RASSF1A hypermethylation and DNA integrity. All these parameters will be quantitatively evaluated by real time PCR, in patients and control subjects. Our data on a preliminary study group of about 70 melanomas and comparable controls indicated that with a sequential quantification [CFDNA, BRAF and RASSF1A] we can obtain a clinical sensitivity of 94% with a specificity of 100%.
Based on these positive preliminary indications, we plan to apply this strategy to a wider clinical study including a large number of patients affected by melanoma and well defined controls. This study should include blood samples from 200 non metastatic patients, at the diagnosis. When possible DNA will be obtained from the paired tissue samples (frozen or FFPE). In melanoma patients blood collection will be repeated one month and one year after surgery.
In this study, we will evaluate whether: i) molecular alterations are detectable in CFDNA from primary cutaneous melanoma patients; ii) the presence of molecular alterations in CFDNA correlates with conventional prognostic histopathological parameters and disease stage; iii) molecular alterations in CFDNA correlate with those observed in primary melanoma tissues; iv) genetic or epigenetic alterations at the time of diagnosis have a prognostic value by correlation with disease-free interval and overall survival on a long-term observation (5 years); v) changes in CFDNA concentrations and appearance of genetic alterations in CFDNA after therapy may represent biomarkers for disease relapse.
The population under investigation will be properly characterized to collect information on relevant risk factors for melanoma and to create a patients' database. This database constitutes a cohort of melanoma cases and a high risk group of subjects that will be followed over time. Subjects in the database will be linked with the Tuscany Cancer Registry and the Tuscany Regional Mortality Registry in order to evaluate if a relation between such multiple markers panel of molecular alterations and disease development and/or progression does exist.
Finally we will assess the feasibility of including in the study a study group formed by outdoor and indoor workers with different patterns of exposure to UV solar radiations.

7. SPECIFIC AIMS (Max 2500 Characters) 2376 car.

The general aim of the proposal is to analyze plasma cell-free DNA (CFDNA) for the detection of genetic and epigenetic alterations in cutaneous melanoma patients, to establish a possible tool for patients' management, specifically in diagnosis, prognosis and follow-up, after therapy.
A multimarker panel of alterations in CFDNA will be designed to guarantee the maximal sensitivity and specificity in melanoma patients. To reach this aim we have preliminarily developed sensitive and accurate assays based on real time PCR that fit the requirement of low detection limit and quantitative information.

This general objective will be accomplished through the following specific aims:

1. To develop quantitative methods to determine the load of DNA alterations in CFDNA and their lower detection limit. This will be crucial to determine cut-off values for the significance of the single DNA alterations.

2. To demonstrate whether a combination of genetic and epigenetic alterations in CFDNA (DNA integrity, mutations of B-RAF, N-RAS, c-KIT and RASSF1A methylation), assessed using highly sensitive methods, may show better sensitivity and specificity for cancer typing.

3. To investigate in melanoma cases and high risk subjects (controls) whether cutaneous melanoma patients show genetic and epigenetic alterations in CFDNA and whether different melanoma subtypes release altered CFDNA at the same rate. It is likely that important differences exist according to anatomical site, main histopathological features (Breslow's thickness, Clark's level, ulceration, tumor growth phase), tumor vascularization, and disease stage. Thus, the predictive value of a particular DNA marker may depend on organ site and tumor type and different DNA mutations might also be markers in different stages of melanoma tumor progression

4. To compare the profile of genetic and epigenetic alterations in tissue specimens derived from the resected melanoma and those observed in CFDNA.

5. To establish whether the level of CFDNA or the presence of a genetic /epigenetic alterations at the diagnosis have a prognostic value by correlation with disease-free interval and overall survival.

6. To evaluate changes in CFDNA concentrations and appearance of genetic alterations in CFDNA in melanoma patients after therapy to assess their potential as markers for disease relapse.

7. To characterize the population under investigation collecting information on relevant risk factors for melanoma and to create a database of cases. This database constitutes a cohort of melanoma cases and a high risk group of subjects that will be followed over time.

8. To evaluate the feasibility of including a control group formed by outdoor and indoor workers characterized by different patterns of exposure to UV solar radiations.

8. BACKGROUND AND RATIONALE (Max 4000 Characters) 3746 car

The incidence of melanoma is steadily increasing within Caucasian population [1]. In Tuscany a significant rise in melanoma incidence and mortality was found in both sexes in the period 1987-2003 [2].

A light phototype, a large number of acquired common nevi [3,4], the presence of atypical nevi [3,4], or a family history of melanoma [5,6] have been associated with an increased risk of melanoma. Melanoma is usually characterized by a high tendency to develop metastasis; thus the most important factor for reducing mortality is early diagnosis, allowing treatment to be undertaken at a stage when cure is readily achievable.
Recent discoveries have allowed for a clearer picture of the molecular events leading to melanoma pathogenesis. The MAPK-ERK pathway (including the cascade of NRAS, BRAF, and MEK1/2 and ERK1/2 gene products) plays a major role in development and progression of melanoma [7,8]. Constitutive activating mutations in NRAS occur in about 20% of melanoma cell lines [9,10], whereas oncogenic BRAF mutations are described in 30–60% of primary melanomas [11-13]. The more frequent BRAF mutation is the V600E, which leads to increased proliferation, invasion and survival of melanoma cells via the activation of MAP kinase pathway.
Melanomas on skin not chronically exposed to sun usually carry either a mutated NRAS or mutated BRAF or concurrently mutated BRAF and PTEN genes [14,15]. BRAF and NRAS mutation are mutually exclusive at the single-cell level providing further support to the hypothesis that expression of the two mutations may not occur in the same neoplastic cell [15]. In contrast, melanomas on skin chronically exposed to the sun or on acral skin, generally present wild-type BRAF or N-RAS genes with subsequent lack of involvement of the RAS–RAF–ERK pathway [16]. Recent data suggest a possible role of c-KIT in subsets of melanoma, including mucosal melanomas (21% mutations), acral cutaneous melanomas (11%) and cutaneous melanomas on skin with chronic sun damage (17%) [16]. This suggests that c-KIT might be of pathogenetic relevance and therefore a therapeutic target in these subtypes of melanoma.
Aberrant methylation of CpG islands of the promoter region of genes was described. The role that this epigenetic event plays in the development of melanoma has recently become an important area of investigation in assessing the mechanisms of tumor suppressor and regulatory gene inactivation. In particular, the methylation of Ras effector homologue RASSF1A promoter is one of the epigenetic changes identified in melanoma.

Circulating nucleic acids represent important biomarkers used as diagnostic and prognostic tools in oncology [17]. Nucleic acids can be associated to circulating tumor cells or free in blood plasma. Cell-free DNA (CFDNA), due to its stability and relative abundance, seems to represent a better tool for clinical application, also providing a non-invasive surrogate for molecular analysis in cancer and pre-cancer patients. CFDNA can be evaluated as total DNA plasma concentration. However, an increased plasma DNA level is not only detectable in tumor patients with cancer or with premalignant states, but also as a consequence of inflammation, trauma and in elderly patients suffering from acute or chronic illnesses [18]. Thus, the availability of innovative techniques able to detect the presence of cancer-associated genetic or epigenetic alterations even in low amount of DNA, make CFDNA as an amenable and a more specific tumor marker.

In addition the identification of melanoma-derived CFDNA and its molecular characterization could represent a key tool for gaining results that may allow a better classification of the different subsets of melanoma patients with different prognosis and, more specifically, the identification of the cellular profiles underlying aggressiveness or responsiveness to therapy.

Genetic or epigenetic alterations have been previously investigated in CFDNA of patients with melanoma.
Most of the previous studies have mainly focused on the qualitative evaluation of a single molecular marker (i.e. presence versus absence of one identifiable alteration in CFDNA) instead of a combination of molecular markers analyzed by a quantitative approach. In this respect, it will be crucial to determine which combination of genetic alterations may carry the best prognostic or diagnostic value in melanoma patients.

9. RESEARCH PLAN (Max 15000 Characters) 12.974 car.

CFDNA represent a promising diagnostic and prognostic biomarker in oncology. In some circumstances, CFDNA alterations are detectable ahead of cancer diagnosis, raising the possibility of exploiting them as biomarkers for monitoring cancer.

Genetic or epigenetic alterations have been previously investigated in CFDNA from melanoma patients. In some instances, these alterations were identical to the ones in the primary tumor tissues, supporting the tumoral origin of altered CFDNA [18]. The detection of melanoma-derived CFDNA, absent or at very low levels in blood of normal individuals, in samples from patients at an early-stage disease could be of paramount importance as indicator of the initiation of dissemination process, which should be preciously recognized to be timely treated. In addition the identification of melanoma-derived CFDNA and its molecular characterization could represent a key tool for gaining results that may allow a better classification of different subsets of melanoma patients with different prognosis and, more specifically, the identification of the cellular profiles underlying aggressiveness or responsiveness to therapy.

Typically, previous case-control studies investigated specific melanoma-derived genetic (i.e. BRAF mutations V600E) [19-22] or epigenetic (RASSF1A methylation) [23] markers. In most cases a single marker was studied separately, in small group of patients and controls apparently not well characterized for parameters like risk of UV exposure, number of nevi, previous skin pathologies, etc. In addition few or no attention was placed on a possible role in early diagnosis of melanoma in population at risk.

Our project aims to evaluate simultaneously a panel of genetic and epigenetic markers (some of them not previously investigated in melanoma) in a large population of patients and well characterized control population. All proposed markers will be evaluated with quantitative procedures. These approaches will allow us to evaluate the results not only in term of positive/negative results, but also to explore the clinical significance of quantification of CFDNA alterations.

The project includes the cooperation of the three Research Units which will indicated as follows:
1st Research Unit (**R.U. 1**) Orlando
2nd Research Unit (**R.U. 2**) Massi
3rd Research Unit (**R.U. 3**) Seniori Costantini.

1. Sample collection from melanoma cases and high risk subjects (controls) (1 – 34 months) (R.U. 2 and 3)

We plan to collect a suitable number of patients covering different clinical conditions, in collaboration with the Department of Dermatology, University of Florence (Research Unit R.U. 2). The study will involve 200 patients with primary melanoma and three control groups (at least 50 subjects for group) with people presenting the main risk factors for the development of melanoma. The first control group will be composed of subjects with less than 50 melanocytic nevi; the second group with more than 100 melanocytic nevi; the third of subjects with a previous history of melanoma, at least 24 months prior. Control group will be stratified according to the age (5-years interval) and sex distribution of the cases.

We will collect three blood samples from each patient with primary melanoma at fixed time interval: at time of surgery, one month and one year later. In the control groups only one blood test will be taken when enrolled in the study. Each blood sampling includes the collection of two separated EDTA tubes (10 ml) as a source of plasma. After centrifugation, DNA will be extracted from plasma using the QIAamp DSP Virus Kit (Qiagen)
In collaboration with Prof. Massi (R.U. 2), we will collect representative fragments of corresponding melanomas from almost all patients undergoing surgery. DNA will be extracted from samples archived as FFPE and used to evaluate the presence of mutations (BRAF, NRAS, c-KIT) or abnormal methylation (i.e. RASFF1A). Finally the correspondence between alterations detectable in

melanoma and plasma-derived CFDNA will be evaluated.
All patients and controls will be asked to sign a written Informed Consent.

2. Quantification of CFDNA (1-34 months) (R. U. 1)
Increased levels of CFDNA were demonstrated in plasma of cancer patients even at an early stage of the tumor development [18]. Even if the absolute concentration of CFDNA cannot be considered as a specific marker, our preliminary data seem to indicate the melanoma patients have significantly higher levels of total plasmatic DNA in comparison to controls subjects. In addition, a correct estimation of CFDNA concentration (ng of DNA/ml plasma) is preliminary to the normalization and the correct quantification of genetically or epigenetically modified DNA sequences.
Since the plasma levels of DNA are undetectable with a conventional spectrophotometric assay, CFDNA concentration will be estimated with real time PCR based on TaqMan probes, referring to the absolute measurement of a single copy gene (APP). A standard curve generated by serial dilutions of normal DNA will allow the measurement of unknown samples.

3. Evaluation of plasma CFDNA integrity (1-34 months) (R. U. 1)
DNA size distribution may be used to determine the origin of CFDNA from either apoptotic or necrotic cells. Tumor necrosis is a frequent event in solid malignant neoplasms and it generates a spectrum of DNA fragments with different strand lengths because of random and incomplete digestion of genomic DNA by a variety of deoxyribonucleases. In contrast, cell death in normal tissues occurs predominantly via apoptosis, resulting in the production of small and uniform DNA fragments [24]. Support for this hypothesis has been found in recent studies demonstrating increased DNA length in plasma from patients with breast and gynaecologic cancers.
Plasma CFDNA integrity was not previously investigated in melanoma patients. We will attempt to use this approach to determine if plasma DNA integrity, measured by DNA strand length, could serve as a potential marker for melanoma and whether altered DNA strand lengths remained in the plasma compartment after surgical resection. We have developed an in-house method that employs a real-time PCR-based assay to assess the DNA strand integrity of plasma DNA. Accordingly, DNA integrity can be defined as the ratio in relative abundance of 306 vs 180 vs 67 bp PCR products of APP gene. This method has already proven to reach the sensitivity and accuracy for the measurement of integrity of CFDNA (1 genome equivalent /ml of plasma).

4. Detection of $BRAF^{V600E}$ in CFDNA(1-34 months) (R. U. 1)
A substantial fraction of melanomas contains a point or tandem oncogenic mutation in exon 15 of BRAF, a cytoplasmic serine/threonine kinase in the MAPK pathway, causing a constitutive activation of B-raf kinase and resulting in downstream activation of the MEK/ERK pathway [25]. Mutations in the BRAF oncogene at amino acid 600 (V600E) have been reported in 40 to 70% of human metastatic melanoma tissues. Previous studies reported also a variable incidence of this mutation in CFDNA of melanoma patients [26].
We developed an allele specific real time PCR to detect mutant BRAF alleles in both primary tumor and CFDNA. The method is highly sensitive and specific and it is based on the use of a pair of primers and a dual labelled LNA probe.

5. Detection of codon 61 mutations of NRAS in CFDNA (6-34 months) (R. U. 1)
Activation of the mitogen-activated protein kinase (MAPK) signalling pathway is found in the majority of melanomas, with either somatic missense mutations of BRAF or, considerably more rarely, mutations of N-RAS. It has long been known that activating NRAS mutations occur in up to 30% of all cutaneous melanoma cases, the most common mutations being in NRAS codon 61. Recently, mutations in NRAS or BRAF were found in 242 of 294 tumours (82%) and were found to be mutually exclusive in all but two cases [27].
For this reason we plan to explore the presence of mutated codon 61 sequences in CFDNA of our patients, to improve the panel of proposed molecular markers. The presence of mutant alleles of codon 61 was not previously explored in melanoma CFDNA. We plan to develop a new method based on High Resolution Melting Analysis for the research of such minoritary alleles.

6. Detection of mutations of c-KIT in exons 9-11 in CFDNA (6-34 months) (R. U. 1)
Mutations and gene amplification in c-KIT have been described in a significant percentage of

mucosal and acral melanomas. Recently, gain-of-function c-KIT mutations were reported in 21% of mucosal melanomas, 11% of acral melanomas, and 16.7% of melanomas arising in chronically sun-damaged skin as indicated by the presence of solar elastosis [28]. Recently, however, Curtin et al., [29] found mutations and/or copy number increases of the KIT in 39% of mucosal melanomas, 36% of acral melanomas and 28% of melanomas on chronically sun-damaged skin, but not in any (0%) melanomas on nonglabrous skin without chronic sun damage. In a separate report, 15% of anal melanomas harboured a KIT oncogenic mutation. Most mutations affect the juxtamembrane region of the KIT protein, which predicts responsiveness to imatinib mesylate. Also in the case of KIT, we have no information regarding the possibility to detect mutated sequence in melanoma CFDNA.
To evaluate the presence of c-KIT copy gain we will develop a classic relative duplex quantitative PCR with TaqMan probes directed to c-KIT sequence and to a typical single copy gene (β-globin). Amplification will be detectable according to the $2^{-\Delta\Delta Ct}$ method.
To detect the presence of mutant alleles of exonss 9 and 11 in CFDNA, we plan to develop a new method based on High Resolution Melting Analysis for the research of such minoritary alleles.

7. Measurement of RASSF1A methylation levels in CFDNA (1-34 months) (R. U. 1)
This gene encodes a protein similar to the RAS effector proteins. Loss or altered expression of this gene has been associated with the pathogenesis of a variety of cancers, which suggests the tumor suppressor function of this gene. The inactivation of this gene was found to be correlated with the hypermethylation of its CpG-island promoter region.
Quantitative Methylation-specific PCR (MSP) is a sensitive and specific assay for tumor-related DNA methylation in CFDNA as demonstrated with studies ion various types of cancer [30].
As regard to the evaluation of epigenetic markers, we will perform Real-time quantitative detection of RASSF1A promoter. DNA will be incubated with the methylation-sensitive enzyme BstUI, to digest unmethylated DNA. Digested DNA will be used for real-time PCR assays for RASSF1A and ß-actin genes quantification. Since ß-actin is an unmethylated gene, it can be used to verify sample complete enzyme digestion represented by promoter undetectable signal. The expected detection limit for methylated RASSF1A is 1 copy/tube.
Potentially, the same approach could be also used for the measurement of other genes potentially hypermethylated in melanoma (i.e., RARB2, MGMT and DAPK and PTEN).

8. Estimation of CFDNA Correlation with tumor burden, stage, clinical outcome and response to therapy (6-36 months) (R. U. 2 and 3)
The results of the analyses will be analyzed with the following end-points:
i) Correlation with melanoma-related conventional clinical and histopathological parameters related to disease Staging, including Breslow thickness, Clark's level and ulceration as well as lymph node status.
ii) Correlation with clinical outcome and prognosis (disease progression and overall survival) and response to therapy.

9. Epidemiological study. Collection of information. (6-36 months) (R.U. 2 and 3)
Molecular epidemiology is interested in measuring a number of biological processes that occur between exposure of the population to risk factors and the final outcome in some individuals, of the disease. In this complex process, CFDNA may be useful as a biomarker at several steps, including the late stages of mutagenesis, clonal expansion, to contributing to early detection of preneoplastic lesions and monitoring of cancer.

In our project, each subject will be interviewed through a standardized questionnaire collecting information on socioeconomic factors, medical history, occupational exposures and detailed information on exposure to UV radiation. Questions will be also asked to obtain information on phenotypical factors. Information on cases and controls will be used in order to investigate differences in the four different groups of subjects under investigations.
Furthermore a database containing all the relevant information collected will be created. Subjects in the database will constitute a cohort of cases that will be followed and linked with the Tuscany Cancer Registry and the Tuscany Regional Mortality Registry in order to evaluate if a relation between such multiple markers panel of molecular alterations and disease progression does exist.
Furthermore the feasibility of including a control group formed by outdoor and indoor workers

characterized by different patterns of exposure to UV solar radiation will be evaluated.

10. Statistical Analysis (6-36 months) (R.U. 3)

Main study objective is to quantify the classification performance of biomarkers derived from circulating DNA fragments. To achieve this goal we will identify different groups of subjects – melanoma cases at different stage, high risk subjects for number of nevi or exposure history. The statistical analysis will follow the line of a generalization of Fisher linear discriminant analysis. First, the classification variables are represented by circulating DNA markers. We will study the multivariate distribution and the appropriateness of Gaussianity assumptions. We will specify a probabilistic model which extend Fisher discrimant analysis to non Gaussian responses and adjust for relevant confounders or design restrictions. In fact, since some subjects characteristics are expected to vary between the study groups – e.g. age distribution between mealanoma patients and subject with high/low nevi count – we will adjust for age imbalance by design. Such sampling constraints must be taken into account in the analysis phase by applying conditional or profile likelihood approaches. [see Chris Fraley; Adrian E Raftery. Model-based clustering, discriminant analysis, and density estimation. Journal of the American Statistical Association; Jun 2002; 97, 458: 611-631.]. Those methods have been developed recently in the context of the analysis of data from Functional Genomics experiments [see for an italian review Gasparini M., Biggeri A. Microarrays and Statistics. Invited lecture Italian Statistical Society, Atti della XL Riunione Scientifica, Milano, 2002].

Secondary aims related to the analysis of the prognostic values of circulating DNA markers in selected populations. The statistical approach is based on Cox's proportional hazard regression model. Since we will assess a multiplicity of potential prognostic variables while adjusting for known factors we have a dimensionality problem. L1 or L2 norm penalized likelihoods will be used [see Tonini G, Baccini M, Cavalieri D, Mini E, Dolara P, Biggeri A. Penalized survival analysis in genome expression experiments. BMC Bioinformatics, (manuscript under revision)].

10. PRELIMINARY RESULTS AND FEASIBILITY (Max 6000 Characters) 4.886 car

Circulating nucleic acids represent important biomarkers used as diagnostic and prognostic tools in oncology.
We performed a preliminary study to evaluate genetic and epigenetic alterations in plasma of melanoma patients, in collaboration with the Department of Dermatological Sciences, of University of Florence (Massi's Research Unit of the present project). The aim of our research was to assess the specificity and sensitivity of four biomarkers for the detection of circulating tumor cells (CTC) and alterations in plasma DNA (CFDNA) of 62 patients affected by melanoma at different stages, 7 patients with non-melanocytic tumors, and 7 subjects with benign melanocytic nevi. The control group included 45 healthy controls. By real time PCR, we assessed the total amount of DNA, by measuring the APP gene, the concentration of DNA containing the $BRAF^{V600E}$ mutation and the methylated sequences of RASSF1A. By real time RT-PCR, we measured tyrosinase (a key enzyme in the synthesis of melanin) mRNA expression.
The parameters showed a significant increase in patients affected by melanoma compared to controls, but no statistical difference was found between control subjects and patients with nevi or non-melanocytic tumors. Clinical sensitivity and specificity were assessed for each parameter, by comparing respective ROC curves. Fixing the specificity value at 100%, the four indicators achieved a diagnostic sensitivity of 65% for APP, 49% for BRAF, 45% for RASSF1A and 25% for the tyrosinase. In particular 34% of patients were positive for only one of the above mentioned markers, 35% for two, 25% for three and only 2% of cases for all the four parameters. Only two patients were negative for all the genetic or epigenetic modifications, with a resulting overall diagnostic sensitivity of 96%. The presence of BRAF mutated alleles in CFDNA was statistically related to the same modification in melanoma.
Our data suggest that the simultaneous determination of a panel of circulating biomarkers in CFDNA (APP+BRAF+RASSF1A) significantly increases the diagnostic sensitivity in cutaneous melanoma. Conversely, the measurement of increased expression of tyrosinase mRNA accounts only for a very limited subset of patients. In addition the procedure for isolation of cancer cell-derived mRNA is cumbersome and affected by serious methodological pitfalls.
Based on these preliminary results and considering the apparent superiority of CFDNA approach, we propose a diagnostic approach based on the sequential determination of APP, BRAF, RASSF1A and other possible targets with assay methods to be developed (i.e. DNA integrity, NRAS and c-KIT mutational hot-spots, hypermethylated promoters of MGMT and DAPK and PTEN genes).
For epidemiological study, the creation of an ad hoc questionnaire, case and control interviews and the database of cases and controls under investigation will be implemented.

Each Research Unit, involved in this project, has a proved expertise in respective areas of interest.

Research Unit 1
The Coordinating Unit (Prof Orlando) operates since several years in cancer diagnosis, mainly through the development and optimization of new molecular tests with potential clinical relevance. The main area of interest was the application of real time PCR and related techniques to the identification of cancer biomarkers in tissue biopsies and in bio-fluids. The group is particularly involved in the study of DNA methylation in cancer.
The Unit of Prof Orlando is equipped with: two 310 Genetic Analyzers; one 7900HT FAST Real-Time PCR system and two TaqMan 7700 Sequence detectors (Applied Biosystems) for real time PCR; a PyroMark ID instrument (Biotage) for pyrosequencing; a Rotor Gene Corbett 3000 for real time PCR; a Rotor Gene Corbett 6000 for real time PCR and HRM; Liquid handling system CAS–1200 Corbett; Bio–Robot EZ1 Qiagen for automatic extraction of nucleic acids; NanoDrop ND–1000 spectrophotometer; Agilent 2100 Bioanalyzer; PALM-ZEISS Laser assisted microdissector.

Research Unit 2
Prof D. Massi has a large competence in diagnosis of melanoma and other skin lesions. She can provide a wide number of archived tissue samples. Dr. V. De Giorgi, is the dermatologist who has the task to collect blood samples from patients and controls. Together, they will collect pathological and clinical data on patients, who will be included in a specific database in a computerized remote

access, privacy securitised data collection system.

Research Unit 3

Dr. Adele Seniori Costantini has a large epidemiological competence, with a particular experience in occupational and environmental epidemiology. The Unit of Occupational and Environmental Epidemiology is involved in the following projects and activities: -Risk and health surveillance and monitoring, -Surveillance studies on environmental risks, -Surveillance studies on occupational risks, -Environmental and occupational analytical studies, -Consulting activities on occupational and environmental risks.

The Unit manages the Tuscan Mortality Registry and the Registries of Occupational cancers (Mesotheliomas and Sino-nasal cancers).

Prof. Annibale Biggeri (Full Professor of Statistics for experimental and technological research, School of Medicine and School of Biotechnology University of Florence; head Biostatistics Unit at the ISPO Cancer Research and Prevention Institute) will provide supervision in the design of the study and the statistical analyses. He co-lead Bioinformatics Work Package in "The European NUTRIGENOMICS organization" (NuGO) NoE VI Framework and he was the national coordinator of two MUIR-PRIN project on Statistical methods in Functional Genomica and System Biology grant 2003133820 and 2005134079 .

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12. PERSONNEL INVOLVED IN THE RESEARCH PROJECT

FIRST AND LAST NAME	Position	Role on Project	Dedicated Time (%)
Claudio Orlando	Associate Professor of Clinical Pathology	Principal Investigator and Unit Scientific Coordinator	40
Mario Pazzagli	Full Professor of Clinical Biochemistry	Real time data analysis	30
Giovanna Danza	Assistant Professor	Molecular analyses	30
Pamela Pinzani	Technician	Molecular analyses	40
Francesca Salvianti	Research Fellow	Molecular analyses	50
To be appointed	Research Fellow	Molecular analyses	100
Daniela Massi	Associate Professor of Pathology	Unit Scientific Coordinator	40
Marco Santucci	Full Professor of Pathology	Morpho-phenotypic characterization of tumor tissues	30
Vincenzo De Giorgi	Dermatologist	Sample collection, Clinical Data and Follow-up	50
Marta Grazzini	Resident in Dermatology	Sample collection, Clinical Data and Follow-up	50
To be appointed	Research Fellow	Sample collection, Clinical Data and Follow-up	100
Adele Rossella Seniori Costantini	Director of occupational and environmental epidemiology –ISPO	Unit Scientific Coordinator	40
Lucia Miligi	Senior Epidemiologist at ISPO	Epidemiological investigation	50
To be appointed	Research Fellow	Database management	100
Annibale Biggeri	Full Professor of Statistics for experimental and technological research	Statistical analysis	20
To be appointed	statistician	Statistical analysis	100

13. CURRICULUM VITAE (Principal Investigator) (Max 4000 Characters)

Degree in Doctor in Biology obtained in 1978, at the University of Florence. Title of PhD in Endocrinology obtained in 1988. Since 1988 Technical Assistant in the Department of Clinical Physiopathology, University of Florence. Since 2001, Assistant Professor, in the same Unit. Since 2005 Associate Professor in Clinical Pathology, at the same Department.
International experiences: Endocrinology and Haematology, Akademisch Ziekenhuis, University' of Gent (Belgium) (1981). Institute of Molecular Biology, Vrije Universiteit of Brussels (Belgium) (1981). Laboratory of Cellular Biology of the University Hospital, Bicetre, Paris (1987). Head of the program for External Quality Control in Molecular Biology for the "Analysis of DNA with PCR amplification" supported by the Italian Society for Clinical Chemistry and Clinical Molecular Biology. Head of the project for External Quality Control in" Quantitative PCR based on TaqMan assay". Head of a section of the National Program "Italian program for quality control in oncology laboratory" supported by the Italian Ministry of Health.
Organizer of 15 training course and scientific meetings. Organizer of the Italian Workshop on Quantitative PCR (9 editions). Author or co-author of more than 100 publications on international journals.
Area of Interest

- Radioimmunoassay of hormones and related clinical applications [*Ann New York Acad Sciences 1990 - Acta Endocrinologica 1992 - J Bone Mineral 1994 - Andrologia 1994*].
- Seminal parameters in male infertility investigation and development of new assay for new biochemical parameters:carnitine, LDH isoenzymes, alfa1-glicosidase e glicerylfosphorilcoline [*Fertil. Steril 1987 - J. Androl. 1988 - Int J Androl 1991 - Int. J. Androl 1994*].
- Bioluminescent techniques for ATP and ADP measurement [*Int. J. Androl. 1982 - Int. J. Androl. 1982 - Metabolism 1994*] and chemiluminescent assay for proteins: transferrin, ceruloplasmin, albumin, somatomedin C [*Fertil. Steril. 1985 - Clin. Chem. 1986 - Methods in Enzimology, 1986 - Int. J. Androl. 1987 - J. Reprod. Fertil. 1988 - Ann New York Acad Sciences 1988 - J. Steroid Biochem. 1989 - J Biolum Chemilum, 1989 - J Steroid Biochem Molec Biol 1992*].
- Immunohystochemical techniques for growth factors in human testis and ovary[*Fertil. Steril. 1986 - Fertil. Steril., 1987 - Fertil. Steril., 1988*].
- Role of endothelin activity in endometrium, parathyroid and testis [*Endocrinology 1990 - J Steroid Biochem Molec Biol 1991 -PNAS, 1991 - PNAS 1991 - Am. J. Physiol. 1992 - Am. J. Physiol. 1993 - Am. J. Physiol. 1995 - J. Androl. 1995 - J Clin Endocrinol Metab. 1996*].
- Quantitative PCR for oncogene amplification and mRNA measurement: [*J. Biolumin. Chemilum. 1994 - Clin Chem, 1994 - Clin Chem. 1995 - Clin. Cancer Res. 1996 - J. Urol 1996 - Clin Chem. 1997 - J Urol 1997 - Clin Cancer Res 1997 - Clin Chem Lab Med 1998 - Clin Chem Lab Med, 1999*]
- Antioxidant capacity in blood samples: methods and applications [*Clin Chem., 1997 - J Biolumin Chemilumin 1998*]
- Development of a new method for telomerase measurement in human cancers [*Clin Chem. 1998*], in bladder cancer diagnosis[*Clin. Cancer Res. 2000 - Urology, 2000*] and in neuroendocrine tumors [*J Clin Endocrinol Metab, 2000*]
- New techniques of quantitative PCR based on real time PCR with the TaqMan system [*Clin Chem 1997- idem 1999 - J Clin Endocrinol Metab 2000 - Regul Peptides 2001 - Clin Chem Lab Med 2001 - Clin Cancer Res 2002 - Anal Biochem 2002 - Clin Chem Lab Med 2003 – J Exp Med 2003 - Eur J Cancer 2003- Endocrinology 2004 – Lung Cancer, 2004 – Endocrinology 2004 - Endocrine-Related Cancer 2004 - Cancer Letters 2004 - Brit J Obst Ginaec 2004 - Clin Chem 2005 - J Clin Endocrinol Metab 2005 - Biochem Biophys Res Commun 2005 - Regul Pept 2005 - Lung Cancer 2006 - Human Pathology 2006 - Oncol Rep 2006 - Clin Exp Metastasis 2006 - Hum Mutat 2007 - Clin Chem Lab Med 2007- Lung Canc 2008 – Melanoma Res 2008 – Am J Path 2008 - Urol Oncol* in press – *Clin Exp Metast* in press*]*

International patent .Plasmids with two or more competitors for competitive PCR techniques'

ISI Papers = 111; Total Impact Factor = 379.1; Mean Impact Factor: 3.41 , Sum of the times cited 2.144; Average citations for items: 13.70; H-Index 24.

14. SELF EVALUATION FORM (Principal Investigator)

Total Papers and Reviews with IF (from January 2003)	46
Total IF (from January 2003)	153.15
Average IF	3.33

Total Papers First/Last Author with IF (from January 2003)	21
Total IF (from January 2003)	62.7
Average IF	2.99

14.1 LIST OF PAPERS WITH IF (FROM JANUARY 2003) OF THE PRINCIPAL INVESTIGATOR

1. Casini Raggi C, Pinzani P, Paradiso A, Pazzagli M, **Orlando C.** *External quality assurance program for PCR amplification of genomic DNA: an Italian experience.* Clin Chem 2003;49:782-791 (I.F. 4.803).
2. Gelmini S, Tricarico C, Petrone L., Forti G, Amorosi A, Dedola GL, Serio M, Pazzagli M, **Orlando C.** Real-Time RT-PCR for the measurement of prostate-specific antigen mRNA expression in benign hyperplasia and adenocarcinomas of prostate. Clin Chem Lab Med 2003; 41:261-265 (I.F. 1.741)
3. Lasagni L, Francalanci , Annunziato f, Lazzeri E, Giannini S, Cosmi L, Sagrinati C, Mazzinghi B, **Orlando C**, Ianni A, Maggi E, Marra F, Romagnani S, Serio M, Romagnani P. *Angiogenesis inibition via a novel type of chemokine receptor generated by alternative splicing of the CXCR3 gene.* J Exp Med 2003, 197:1537-1549 (I.F. 15.612)
4. Mori M, Manuelli C, Pimpinelli N, Bianchi B, **Orlando C**, Mavilia C, Cappugi, P, Maggi E, Giannotti B, Santucci M. *BCA-1, A B-cell chemoattractant signal, is constantly expressed in cutaneous lymphoproliferative B-cell disorders.* Eur J Cancer 2003;39:1625-31 (I.F. 4.454).
5. Cameron Smith M, **Orlando C**, Serio M, Maggi M. *Somatostatin receptors and breast cancer.* J Endocrinol Invest. 2003;26:125-30. Review. (I.F. 2.021).
6. Vignozzi L, Filippi S, Luconi M, Morelli A, Mancina R, Marini M, Vannelli GB, Granchi S, **Orlando C**, Gelmini S, Ledda F, Forti G, Maggi M. *Oxytocin receptor is expressed in the penis and mediates an estrogen-deependent smooth muscle contractility.* Endocrinology. 2004, 145:1823-34 (I.F. 5.045)
7. Morelli A, Filippi S, Mancina R, Luconi M, Vignozzi L, Marini M, **Orlando C**, Vannelli GB, Aversa A, Natali A, Forti G, Giorgi M, Jannini EA, Ledda F, Maggi M. *Androgens regulate phosphodiesterase type 5 expression and functional activity in corpora cavernosa.* Endocrinology 2004;145:2253-63 (I.F. 5.045)
8. Simi L, Andreani M, Davini F, Janni A, Pazzagli M, Serio M, **Orlando C.** *Simultaneous measurement of MMP9 and TIMP1 mRNA in human non small cell lung cancers by multiplex real time RT-PCR.* Lung Cancer 2004, 2004;45:171-9 (I.F. 3.455)
9. **Orlando C,** Casini Raggi C, Bianchi S, Distante V, Simi L, Vezzosi V, Gelmini S, Pinzani P, Cameron Smith M, Buonamano A, Lazzeri E, Pazzagli M, Cataliotti L, Maggi M, Serio M. *Measurement of Somatostatin Receptor Subtype 2 mRNA in Breast Cancer and Corresponding Normal Tissue.* Endocrine-Related Cancer, 2004;11:323-32 (I.F. 5.193)
10. Verderio P, **Orlando C**, Casini Raggi C, Marubini E. *Confidence interval estimation for DNA and mRNA concentration by real-time PCR: A new environment for an old theorem.* Int J Biol Markers. 2004;19:76-9 (I.F. 1.208)
11. Smith MC, Maggi M, **Orlando C.** *Somatostatin receptors in non-endocrine tumours.* Dig Liver Dis. 2004;36:S78-85 (I.F. 1.982)
12. S Gelmini, M Poggesi, V Distante,S Bianchi, L Simi, M Luconi, C Casini Raggi, L Cataliotti, M Pazzagli, **C Orlando.** *Tankyrase, a positive regulator of telomere elongation,is over expressed in human breast cancer.* Cancer Letters. 2004;216:81-7 (I.F. 3.398)
13. Marubini E, Verderio P, Raggi CC, Pazzagli M, **Orlando C.** *Statistical diagnostics emerging from external quality control of real-time PCR.* Int J. Biol. Markers. 2004;19:141-6 (I.F. 1.208).
14. Sgambati E, Marini M, Zappoli Thyrion GD, Parretti E, Mello G, Orlando C, Simi L, Tricarico C, Gheri G, Brizzi E. *VEGF expression in the placenta from pregnancies complicated by*

hypertensive disorders. Brit J Obst Ginaec. 2004;111:564-70 (I.F. 2.196).
15. Filopanti M, Ballare E, Lania AG, Bondioni S, Verga U, Locatelli M, Zavanone LM, Losa M, Gelmini S, Peri A, Orlando C, Beck-Peccoz P, Spada A. *Loss of heterozygosity at the SS receptor type 5 locus in human GH- and TSH-secreting pituitary adenomas*. J Endocrinol Invest. 2004;27:937-42 (I.F. 2.636)
16. Salvadori B, Pinzani P, Distante V, Casella D, Bianchi S, Paglierani M, Vezzosi V, Neumann R, Cataliotti L, Pazzagli M, **Orlando C**. *Comparison of pre- and postsurgical concentrations of blood HER-2 mRNA and HER-2 extracellular domain reflects HER-2 status in early breast cancer.* Clin Chem. 2005;51:254-6 (I.F. 4.803).
17. C Casini Raggi, P Verderio, M Pazzagli, E Marubini, L Simi, P Pinzani, A Paradiso, **C Orlando**. *An Italian Program of External Quality Control for Quantitative Assays Based on Real-Time PCR with Taq-Man□ Probes*. Clin Chem Lab Med 2005; 43:542-8 (I.F. 1.741)
18. Luchi N, Capretti P, Pinzani P, **Orlando C**, Pazzagli M. *Real-time PCR detection of Biscogniauxia mediterranea in symptomless oak tissue*. Lett Appl Microbiol. 2005;41:61-8 (I.F. 1.623)
19. Filopanti M, Ronchi C, Ballare E, Bondioni S, Lania AG, Losa M, Gelmini S, Peri A, **Orlando C**, Beck-Peccoz P, Spada A. *Analysis of somatostatin receptor 2 and 5 polymorphisms in patients with acromegaly*. J Clin Endocrinol Metab. 2005; 90:4824-8 (I.F. 5.493)
20. Malentacchi F, Marzocchini R, Gelmini S, **Orlando C**, Serio M, Ramponi G, Raugei G. *Up-regulated expression of low molecular weight protein tyrosine phosphatases in different human cancers.* Biochem Biophys Res Commun. 2005;334:875-83. (I.F. 2.749)
21. Raggi CC, Cianchi F, Valanzano R, Smith MC, Serio M, Maggi M, **Orlando C**. *Prognostic value of somatostatin receptor subtype 2 expression in colorectal cancer*. Regul Pept. 2005;132:23-6. (I.F. 2.422)
22. Gelmini S, Malentacchi F, Giannarini GL, Selli C, **Orlando C**. *New insights in telomerase and telomeric proteins and their applicationsto urological cancers.* European Association Urology Supplement 2005;3:189-199 (I.F. 2.507)
23. Reina G, **Orlando C**, Rebora P, Ambrogi F, Casini Raggi C, Verderio P, Marubini E. *Bivariate statistical approach to evaluate laboratory performance by analysis of standard curves in an External Quality Assurance program for quantitative assays based on real-time PCR with Taq-Man probes*. Clin Chem Lab Med. 2006; 44:18-22 (I.F. 1.741)
24. Simi L, Venturini G, Malentacchi F, Gelmini S, Andreani M, Janni A, Pastorekova S, Supuran C, Pazzagli M, **Orlando C**. *Quantitative analysis of carbonic anhydrase IX mRNA in human non small cell lung cancer*. Lung Cancer 2006, 52:59-66 (I.F. 3.455)
25. Pinzani P, **Orlando C**, and Pazzagli M. *Laser assisted microdissection for real-time PCR sample preparation*. Molecular Aspects of Medicine 2006; 27:140-159 (I.F. 7.386)
26. Pinzani P , Salvadori B, Simi L, Bianchi S, Distante V, Cataliotti L, Pazzagli M, **Orlando C.** *Isolation by Size of Epithelial Tumor Cells (ISET) in Peripheral Blood of Breast Cancer Patients. Correlation with real-time RT-PCR results and feasibility of molecular analysis by laser microdissection.* Human Pathology, 2006;37:711-8 (I.F. 3.034)
27. Gelmini S, Poggesi M, Pinzani P, Mannurita SC, Cianchi F, Valanzano R, **Orlando C.** *Distribution of Tankyrase-1 mRNA expression in colon cancer and its prospective correlation with progression stage*. Oncol Rep. 2006;16:1261-6. (I.F. 1.597)
28. Prignano F, Gerlini G, Salvatori B, **Orlando C**, Mazzoli S, Pimpinelli N, Moretti S. *Stem cell factor affects tumour progression markers in metastatic melanoma cells.* Clin Exp Metastasis. 2006;23:177-86 (I.F. 2.514)
29. Ramsden SC, Daly S, Geilenkeuser WJ, Duncan G, Hermitte F, Marubini E, Neumaier M, **Orlando C**, Palicka V, Paradiso A, Pazzagli M, Pizzamiglio S, Verderio P. *EQUAL-quant: an international external quality assessment scheme for real-time PCR*. Clin Chem. 2006;52:1584-9, 2006 (I.F. 4.803)
30. Caciotti A, Donati MA, Procopio E, Filocamo M, Kleijer W, Wuyts W, Blaumeiser B, d'Azzo A, Simi L, **Orlando C**, McKenzie F, Fiumara A, Zammarchi E, Morrone A. *GM1 gangliosidosis: molecular analysis of nine patients and development of an RT-PCR assay for GLB1 gene expression profiling*. Hum Mutat. 2007; 28 :204 (I.F. 6.273)
31. Gelmini S, Quattrone S, Malentacchi F, Villari D, travaglino F, Giannarini G, DellaMelina A, Pazzagli M, Nicita G, Selli C, **Orlando C**. *Tankyrase-1 mRNA expression in bladder cancer and paired urine sediment: preliminary experience*. Clin Chem Lab Med. 2007; 45:862-6 (I.F. 1.741)

32. **Orlando C**, Verderio P, Maatman R, Danneberg J, Ramsden S, Neumaier M, Taruscio D, V Falbo V, Jansen R, Casini-Raggi C, Malentacchi F, Marubini E, Pizzamiglio S, Vernelen K, Libere JC, Palicka V, Pazzagli M. *EQUAL-qual: A European Program for External Quality Assessment of genomic DNA extraction and PCR Amplification*. Clin Chem. 2007; 53:1349-57 (I.F. 4.803)
33. Simi L, Pinzani P, Raggi CC, Pazzagli M, **Orlando C**. *Influence of 17q gain and promoter polymorphisms on mRNA expression of somatostatin receptor type 2 in neuroblastoma.*Clin Chim Acta. 2007;384:149-54 (I.F. 2.601)
34. Cavalieri D., Dolara P., Mini E., Luceri C., Castagnini C, Toti S, Maciag K, De Filippo C, Nobili S, Morganti M , Napoli C, Tonini G, Baccini M, Biggeri A, Tonelli F, Valanzano R, **Orlando C**, Gelmini S, Cianchi F, Messerini L, Luzzatto L. *Analysis of gene expression profiles reveals novel correlations with the clinical course of colorectal cancer*. Oncol. Res. 2007;16:535-48 (IF. 1.347)
35. Pizzamiglio S, Verderio P, **Orlando C**, Marubini E. *Confidence interval for DNA/mRNA concentration by real-time PCR. Int J Biol Markers*. 2007;22:232-6 (I.F. 1.208).
36. Fiotti N, Altamura N, **Orlando C**, Simi L, Reimers B, Pascotto P, Zingone B, Pascotto A, Serio M, Guarnieri G, Giansante C. *Metalloproteinases-2, -9 and TIMP-1 expression in stable and unstable coronary plaques undergoing PCI.* Int J Cardiol. 2007; 127:350-7 (I.F. 2.878)
37. Verderio P, Ramsden SC, **Orlando C**, Pizzamiglio S, Paradiso A, Neumaier M, Pazzagli M, Marubini E. *External quality assessment schemes for real-time PCR: a statistical procedure to corrective actions.* Clin Chem Lab Med. 2008, 46:717-21. (I.F. 1.741)
38. Sestini R, Provenzano A, Bacci C, **Orlando C**, Genuardi M, Papi L. *NF2 mutation screening by Denaturing High Performance Liquid Chromatography and High Resolution Melting Analysis*. Genet Test, 2008, 12:311-8 (IF 1.218)
39. Pinzani P, Lind K , Malentacchi F, Nesi G, Salvianti F, Kubista M, Pazzagli M, **Orlando C**. *Real-time RT-qPCR and immuno-qPCR for the simultaneous measurement of PSA mRNA and PSA protein in laser microdissected cells of human prostate*. Human Pathology 2008, 39:1474-82 (IF. 3.034)
40. Simi L, Pratesi N, Vignoli M, Sestini R, Cianchi F,Valanzano R, Nobili S, Mini E, Pazzagli M, **Orlando C.** *High resolution melting analysis for rapid detection of KRAS, BRAF and PIK3CA gene mutations in colorectal cancer*. Am J Clin Path 2008, 130 :247-53. (IF 2.629)
41. Carraresi L, Parini R, Filoni C, Caciotti A, Sersale G, Tomatsu S, **Orlando C**, Zammarchi E, Guerrini R, Donati MA, Morrone A. *GALNS gene expression profiling in Morquio A patients' fibroblasts.* Clin Chim Acta. 2008; 397:72-6. (IF 2.601)
42. Malentacchi F, Simi L, Nannelli C, Andreani M,Janni A, Pastorekova S, **Orlando C.** *Alternative splicing variants of carbonic anhydrase IX in human non-small cell lung cancer*. Lung Cancer, 2008 in press (IF 3.455)
43. Vignoli M, Scaini MC, Ghiorzo P, Sestini R, Bruno W, Menin C, Gensini F, Piazzini M, Testori A, Manoukian S, **Orlando C**, D'Andrea E, Bianchi-Scarrà G, Genuardi M. *Genomic rearrangements of the CDKN2A locus are infrequent in Italian malignant melanoma families without evidence of CDKN2A/CDK4 point mutations.* Melanoma Res. 2008;18:431-437 (IF 2.225)
44. F Di Costanzo, P Pinzani, **C Orlando**, S Gasperoni, L Vannini, L Antonuzzo, M Pazzagli. *Circulating tumour cells in colorectal cancer.* Eur J Cancer, 2008;6:52-59 (I.F. 4.454)
45. Vinci S, Selli C, Kuncova J, Villari D, **Orlando C.** *Quantitative Methylation Analysis of Bcl2, hTERT and DAPK Genes in Urine Sediment as a Tool in the Diagnosis of Superficial Transitional Cell Carcinomas of the Urinary Bladder*. Urol Oncol, in press (I.F. 2.561)
46. S Gelmini, M Mangoni, F Castiglione, C Beltrami, A Pieralli, KL Andersson, M Fambrini, GL Taddei, M Serio, **C Orlando**. *The CXCR4/CXCL12 axis in endometrial cancer*. Clin Exp Met, in press (I.F. 2.514)

15. LIST OF PAPERS WITH IF (FROM JANUARY 2003) OF THE SCIENTIFIC COORDINATOR(S) OF ADDITIONAL RESEARCH UNIT(S)

Massi Daniela - publications

1. Santucci M., Pimpinelli N., **Massi D.**, Kadin M.E., Meijer C.J.L.M., Müller–Hermelink H.K., Paulli M., Wechsler J., Willemze R., Audring H., Bernengo M.G., Cerroni L., Chimenti S., Chott A., Díaz-Pérez J.L., Dippel E., Duncan L.M., Feller A.C., Geerts M.L., Hallermann C., Kempf W., Russell-Jones R., Sander C., Berti E. Cytotoxic/Natural Killer cell cutaneous lymphomas: Report of the E.O.R.T.C. cutaneous lymphoma task force Workshop. *Cancer* 2003;97:610-27. IF 4.434
2. **Massi D.**, Franchi A., Ketabchi S., Paglierani M., Pimpinelli N., Santucci M. Expression and prognostic significance of matrix metalloproteinases and their tissue inhibitors in patients with primary neuroendocrine carcinoma of the skin. *Hum Pathol* 2003;34:80-8. IF 3.369
3. De Giorgi V., **Massi D.**, Gerlini G., Mannone F., Quercioli E., Carli P. Immediate local and regional recurrence after the excision of a polypoid melanoma: Tumour dormancy or tumor activation? *Dermatol Surg* 2003;29:664-7. IF 2.309
4. De Giorgi V., **Massi D.**, Trez E., Salvini C., Quercioli E., Carli P. Blue hue in dermoscopy setting: homogeneous blue pigmentation, gray-blue area or whitish blue veil? *Dermatol Surg* 2003;29:965-7. IF 2.309
5. Carli P., Balzi D., De Giorgi V., **Massi D.**, Palli D., Chiarugi A., Nardini P., Giannotti B. Results of surveillance programme aimed at early diagnosis of cutaneous melanoma in high risk Mediterranean subjects. *Eur J Dermatol* 2003;13:482-6. IF 1.303
6. Franchi A., Baroni G., **Massi D.**, Santucci M. CDX-2 homeobox gene expression. *Am J Surg Pathol* 2003;27:1390-1. IF 4.690
7. Masciullo V., Susini T., Zamparelli A., Bovicelli A., Minimo C., **Massi D.**, Taddei G.L., Maggiano N., De Iaco P., Ceccaroni M., Bovicelli L., Massi G.B., Mancuso S., Scambia G., Giordano A. Frequent Loss of Expression of the Cyclin-dependent Kinase Inhibitor p27kip1 in Endometrial Cancer: Lack of Prognostical Significance. *Clin Cancer Res* 2003;9:5332-8. IF 6.177
8. De Giorgi V., **Massi D.**, Trez E., Alfaioli B., Carli P. Multiple pigmented trichoblastomas and syringocystoadenoma papilliferum in nevus sebaceous mimicking a malignant melanoma: A clinical dermoscopic-pathological case study. *Br J Dermatol* 2003;149:1067-70. IF 3.334
9. De Giorgi V., **Massi D.**, Sestini S., Alfaioli B, Carli P. Elderly woman with rapidly growing, ulcerated pipgmented lesion. *Can Med Assoc J* 2003;169:1054. IF 6.862
10. Stante M., De Giorgi V., **Massi D.**, Chiarugi A., Carli P. Pigmented Bowen's disease mimicking cutaneous melanoma: Clinical and dermoscopic aspects. *Dermatol Surg* 2004;30:541-4. IF 2.309
11. **Massi D.**, Franchi A., Paglierani M., Ketabchi S., Borgognoni L., Reali U.M., Santucci M. Vasculogenic mimicry has no prognostic significance in pT3 and pT4 cutaneous melanoma. *Hum Pathol* 2004;35:496-502. IF 3.369
12. Carli P., De Giorgi V., Crocetti E., Mannone F., **Massi D.**, Chiarugi A., Giannotti B. Improvement of malignant/benign ratio in excised melanocytic lesions in the "dermoscopy era": A retrospective study 1997-2001. *Br J Dermatol* 2004;150:687-92. IF 3.334
13. Moretti S., Amato L., **Massi D.**, Bianchi B., Gallerani E., Fabbri P. Evaluation of inflammatory infiltrate and fibrogenic cytokines in pseudopelade of Brocq suggests the involvement of Th2 and Th3 cytokines. *Br J Dermatol* 2004;151:84-90. IF 3.334
14. **Massi D.**, Beltrami G., Mela M.M., Pertici M., Capanna R., Franchi A. Prognostic factors in soft tissue leiomyosarcoma of the extremities: A retrospective analysis of 42 cases. *Eur J Surg Oncol* 2004;30:565-572. IF 1.882
15. De Giorgi V., **Massi D.**, Salvini C., Trez E., Mannone F., Carli P. Dermoscopic features of combined melanocytic nevi. *J Cutan Pathol 2004;*31:600-4. IF 1.582
16. Fabbri P., Amato L., Chiarini C., Moretti S., **Massi D.** Scarring alopecia in discoid lupus erythematosus: a clinical, histopathologic and immunopathologic study. *Lupus* 2004;13:455-462. IF 2.366
17. Franchi A., **Massi D.**, Palomba A., Biancalani M. Santucci M. CDX-2, cytokeratin 7 and cytokeratin 20 immunohistochemical expression in the differential diagnosis of primary adenocarcinomas of the sinonasal tract. *Virchows Arch* 2004;445:63-7. IF 2.251

18. Franchi A., Gallo O., **Massi D.**, Baroni G., Santucci M. Tumor lymphangiogenesis in head and neck squamous cell carcinoma. A morphometric study with clinical correlations. *Cancer* 2004;101:973-8. IF 4.434
19. Mannone F., De Giorgi V., Cattaneo A., **Massi D.**, De Magnis A., Carli P. Dermoscopical features of mucosal melanosis. *Dermatol Surg* 2004;30:1118-23. IF 2.309
20. **Massi D.**, Beltrami G., Capanna R., Franchi A. Histopathological re-classification of extremity pleomorphic soft tissue sarcoma has clinical significance. *Eur J Surg Oncol* 2004;30:1131-6. IF 1.882
21. De Giorgi V., Stante M., **Massi D.**, Mavilia L., Cappugi P., Carli P. Possible histopathologic correlates of dermoscopic features in pigmented melanocytic lesions by means of optical coherence tomography. *Exp Dermatol* 2005;14:56-59. IF 2.449
22. De Giorgi V., **Massi D.**, Sestini S., Alfaioli B., Carelli G., Carli P. Cutaneous collision tumour (melanocytic naevus, basal cell carcinoma, seborrhoeic keratosis): A clinical, dermoscopic and pathological case report. *Br J Dermatol* 2005;152:787-90. IF 3.334
23. De Giorgi V., Salvini C., **Massi D.**, Raspollini M.R., Carli P. Vulvar basal cell carcinoma: retrospective study and review of literature. *Gynecol Oncol* 2005;97:192-194. IF 2.083
24. Cicchi R., Pavone F., **Massi D.**, Sampson DD. Contrast and depth enhancement in two-photon microscopy of human skin ex vivo by use of optical clearing agents. *Opt Express* 2005;13:2337-44. IF 4.009
25. **Massi D.**, Naldini A., Ardinghi C., Carraro F., Franchi A., Paglierani M., Tarantini F., Ketabchi S., Geppetti P., Hollenberg M.D., Cirino G., Santucci M. Expression of protease-activated receptor-1 and -2 in melanocytic nevi and human cutaneous melanoma. *Hum Pathol* 2005;36:676-85. IF 3.369
26. De Giorgi V., Salvini C., **Massi D.**, Sestini S., DiFonzo E.M., Carli P. Ungual basal cell carcinoma of the fifth toe mimicking chronic dermatitis. *Dermatol Surg* 2005;31:723-5. IF 2.309
27. Berti S., Moretti S., Lucin C., Amato L., **Massi D.**, Fabbri P. Urticarial vasculitis and subacute cutaneous lupus erythematosus. *Lupus* 2005;14:489-92. IF 2.366
28. De Giorgi V., **Massi D.**, Salvini C., Mannone F., Carli P. Thin melanoma of the vulva: a clinical, dermoscopic-pathologic case study. *Arch Dermatol* 2005;141:1046-7. IF 2.851
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18. Veraldi A, **Seniori Costantini A**, Bolejack V, Miligi L, Vineis P, van Loveren H. Immunotoxic effects of chemicals: A matrix for occupational and environmental epidemiological studies. Am J Ind Med. 2006 Dec;49(12):1046-55. **IF** 1.597
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16. FINANCIAL REQUEST

16.1 PRINCIPAL INVESTIGATOR'S UNIT (S)

	Year 1	Year 2	Year 3	Total
NON-STAFF PERSONNEL (CONTRACTS, FELLOWSHIPS, ETC). SPECIFY: FULL-TIME FELLOWSHIP FOR A MOLECULAR BIOLOGIST/BIOTECHNICIAN	19000	19000	19000	57000
CONSUMABLES SUPPLIES: REAGENTS FOR NUCLEICIC ACID EXTRACTION, PCR, SEQUENCING, METHYLATION STUDIES, PROBES, PRIMERS AND PLASTIC	42000	42000	42000	126000
SMALL EQUIPMENT(S) SPECIFY:				
TRAVEL EXPENSES (MEETINGS, COURSES, ETC.)	1000	1000	1000	3000
PUBBLICATION COSTS. SPECIFY:				
OVERHEAD.	6000	6000	5000	18000
TOTAL COSTS	68000	68000	68000	204000

16.2 ADDITIONAL UNIT(S) WITHIN REGIONE TOSCANA MASSI UNIT

	Year 1	Year 2	Year 3	Total
NON-STAFF PERSONNEL (CONTRACTS, FELLOWSHIPS, ETC). SPECIFY:	19000	19000	19000	57000
CONSUMABLES SUPPLIES:	5000	5000	5000	15000
SMALL EQUIPMENT(S) (MAX 20.000 EUROS). SPECIFY:				
TRAVEL EXPENSES (MEETINGS, COURSES, ETC.)	3000	3000	3000	9000
PUBBLICATION COSTS. SPECIFY:	2000	2000	2000	6000
OVERHEAD.	3000	3000	3000	9000
TOTAL COSTS	32000	32000	32000	96000

16.2 ADDITIONAL UNIT(S) WITHIN REGIONE TOSCANA
SENIORI COSTANTINI UNIT

NON-STAFF PERSONNEL (CONTRACTS, FELLOWSHIPS, ETC). SPECIFY: **FELLOSHIP FOR AN EPIDEMIOLOGIST AND A STATISTICIAN**	**20000**	**20000**	**20000**	**60000**
CONSUMABLES SUPPLIES:				
SMALL EQUIPMENT(S) (MAX 20.000 EUROS). SPECIFY:				
TRAVEL EXPENSES (MEETINGS, COURSES, ETC.)	**2500**	**2500**	**2500**	**7500**
PUBBLICATION COSTS. SPECIFY:				
OVERHEAD. 20%	**2500**	**2500**	**2500**	**7500**
TOTAL COSTS	**25000**	**25000**	**25000**	**75000**

16.3 TOTAL

	Year 1	Year 2	Year 3	Total
TOTAL COSTS	**125000**	**125000**	**125000**	**375000**

17. AVAILABLE GRANT(S) CO-FINANCING THE PROPOSAL (Principal Investigator)

Project Title	**Integrated Project in Oncology**: Analytical and clinical validation of new biomarkers for early diagnosis: the Network, the resources, the metodology, the QC, the analysis of data
Principal Investigator	Dr Angelo Paradiso
Granting Agency	Italian Ministry of Health
Amount Granted (Euros)	30.000
Amount Available for Co-Financing (Euros)	5.000

Project Title	**SPIDIA project** ("*Standardisation and improvement of generic Pre-analytical tools and procedures for In-vitro DIAgnostics*")
Principal Investigator	Prof Mario Pazzagli
Granting Agency	European 7th Framework Programme
Amount Granted (Euros)	500.000
Amount Available for Co-Financing (Euros)	25.000

17.1 AVAILABLE GRANT(S) CO-FINANCING THE PROPOSAL (Additional Research Units)

MASSI UNIT

Project Title	Tumori cutanei e fotoinvechiamento: applicazione di metologie ottiche innovative per una diagnosi precoce e gestione del paziente dal laboratorio alla clinica specializzata
Principal Investigator	Daniela Massi - Torello Lotti
Granting Agency	Fondazione Ente Cassa di Risparmio
Amount Granted (Euros)	110000
Amount Available for Co-Financing (Euros)	30000

18. SUGGESTED REVIEWERS (MAX 3).

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E-MAIL	m.cook@nhs.net

FIRST AND LAST NAME	**HENRI MONDOR - REZ-DE-CHAUSSEE**
POSITION	
INSTITUTION	ANATOMIE PATHOLOGIQUE
ADDRESS	51 AVENUE DU MAL DE TASSIGNY
CITY	94010 CRETEIL CEDEX FRANCE
PHONE	
E-MAIL	jwechsler@noos.fr

19. BIO-ETHICAL REQUIREMENTS

Does the proposed research involve:

HUMAN EXPERIMENTATION YES X NO

Human experimentation includes involvement of human subjects and other issues with ethical implications.

If YES include clearance from the competent Ethical Committee (as addendum E). If approval of the Ethical Committee is not available at moment of submission, include as addendum E, a **signed** statement from the proponent pledging to obtain it before the start of the research. ITT will not allocate funds in the absence of an ethical committee clearance.

ANIMAL EXPERIMENTATION YES NO X

If YES sign the following.

The Ethical Committee for animal use in cancer research has evaluated the proposal.
The Committee considers tht the preliminary results, obtained in in-vitro experiments deserve a preclinical study.
The procedures related to animal use are accurately described in the proposal and conform to all regulations protecting animals used for research purposes, including those of the DL 116/92. The experiments described in the proposal will be performed following the detailed Internal Regulation drawn-up according to: Workamm P., et al. (1998) United Kingdom Coordinating Committee on Cancer Research (Guidelines for the welfare of animals in experimental neoplasia. Br. J. Cancer 77: 1-10).

Date:	Name of PI:	Signature:
19.2.2009	Claudio ORLANDO	

20. DECLARATION AND PRIVACY STATEMENT

I hereby certify that all information submitted in the application form is accurate and complete.
I agree that, in accordance with law 196/2003 , the processing of my personal data shall be performed for the following purposes:

1. administrative management of the dossiers;
2. evaluation of the value of the research projects with transmission of the data to the *Italian and non-Italian referees/evaluators;*
3. activities ancillary and/or pursuant to the above;

The communication of personal data for these purposes in not compulsory although refusal to do so, owing to the peculiarity of the relationship between the data and the aim for which it is requested, will render the candidate ineligiblefor selection/award.

Date:

19.2.2009

Signature:

Claudio ORLANDO

1. RIMODULAZIONE

All 3

ISTITUTO TOSCANO TUMORI (ITT) – REGIONE TOSCANA
GRANT PROPOSAL 2008

1. PRINCIPAL INVESTIGATOR (PI)

First and last Name	Claudio ORLANDO
Position	Associate Professor
Institution	Department of Clinical Physiopathology
Address	viale Pieraccini 6
City	Florence
Phone	+39.0554271440
E-mail	c.orlando@dfc.unifi.it
Legal representative	Prof. Augusto Marinelli, Rector of the University of Florence
Address	Pzza San Marco 4
E-mail	rettore@unifi.it

2. PROJECT TITLE (Max 150 Characters)

Identification and Clinical Evaluation of Genetic and Epigenetic Multiple Markers in Cell-Free Plasma DNA of Melanoma Patients

16. FINANCIAL REQUEST

16.1 PRINCIPAL INVESTIGATOR'S UNIT (S)

	Year 1	Year 2	Year 3	Total
Non-Staff Personnel (Contracts, Fellowships, etc). Specify: Full-time fellowship for a molecular biologist/biotechnician	14000	14000	14000	42000
Consumables supplies: reagents for nucleicic acid extraction, PCR, sequencing, methylation studies, probes, primers and plastic	21000	13000	16000	50000
Small Equipment(s) Specify:				
Travel Expenses (Meetings, Courses, etc.)	1000	1000	1000	3000
Pubblication Costs. Specify:				
Overhead.	3000	3000	3000	9000
Total Costs	39000	31000	34000	104000

16.2 ADDITIONAL UNIT(S) WITHIN REGIONE TOSCANA
MASSI UNIT

	Year 1	Year 2	Year 3	Total
NON-STAFF PERSONNEL (CONTRACTS, FELLOWSHIPS, ETC). SPECIFY:	15000	5000	5000	25000
CONSUMABLES SUPPLIES:	3500	3000	2000	8500
SMALL EQUIPMENT(S) (MAX 20.000 EUROS). SPECIFY:				
TRAVEL EXPENSES (MEETINGS, COURSES, ETC.)	2000	1500	1500	5000
PUBBLICATION COSTS. SPECIFY:		1500		1500
OVERHEAD.	1500	1500	1000	4000
TOTAL COSTS	22000	12500	9500	44000

16.2 ADDITIONAL UNIT(S) WITHIN REGIONE TOSCANA
SENIORI COSTANTINI UNIT

NON-STAFF PERSONNEL (CONTRACTS, FELLOWSHIPS, ETC). SPECIFY: FELLOSHIP FOR AN EPIDEMIOLOGIST AND A STATISTICIAN		15000	15000	30000
CONSUMABLES SUPPLIES:				
SMALL EQUIPMENT(S) (MAX 20.000 EUROS). SPECIFY:				
TRAVEL EXPENSES (MEETINGS, COURSES, ETC.)				
PUBBLICATION COSTS. SPECIFY:				
OVERHEAD.		1500	1500	3000
TOTAL COSTS	0	16500	16500	33000

16.3 TOTAL

	Year 1	Year 2	Year 3	Total
TOTAL COSTS	61000	60000	60000	181000

ALLEGATO "B" ALLA DELIBERA D.G. N. 111 DEL 7/09/2012

ISTITUTO PER LO STUDIO
E LA PREVENZIONE ONCOLOGICA

Servizio Sanitario della Toscana

Firenze, 11/9/12

Al Direttore Generale
ISPO
Al Direttore Sanitario
ISPO

Oggetto: Relazione al progetto "Identification and Clinical Evaluation of Genetic and Epigenetic Multiple Markers in Cell-Free Plasma DNA of Melanoma Patients" coordinato dal Prof. Claudio Orlando del Dipartimento di Fisiopatologia Clinica dell' Università di Firenze – finanziato dall'Università di Firenze.

Obiettivo principale del progetto è quello di studiare alterazione molecolari in pazienti con melanoma cutaneo e su un campione di controlli.
Nell' ambito del progetto il ruolo dell' ISPO è relativo ad un approfondimento epidemiologico e all' analisi statistica, in particolare la *SC di Epidemiologia Ambientale ed Occupazionale* sarà coinvolta nel raccogliere informazioni su un campione dei soggetti in studio – soggetti afferenti all' ambulatorio ISPO sul melanoma - in particolare su informazioni di tipo socio economico, informazione cliniche, informazioni sulla storia lavorativa con particolare riguardo all'esposizione a radiazione solare ultravioletta , esposizione ricreativa o medica a radiazione UV. Verranno inoltre raccolte informazioni su fenotipo e fototipo. Le informazioni raccolte verranno informatizzate creando un database dei soggetti in studio, unitamente a quelli che affluiscono ad altre dermatologie- che potrà costituire una coorte di soggetti che potrà essere seguita nel tempo. Il link dei soggetti in studio con l'archivio di mortalità regionale (RMR) e con il Registro Toscano tumori (RTT) potrà dare informazioni sulla relazione tra le alterazioni molecolari osservate e la progressione della malattia. Sarà inoltre valutata la possibilità di includere tra i soggetti un gruppo di lavoratori outdoor e indoor.
Per il suddetto progetto sarà necessario acquisire personale (Assistente Sanitario, Biologo) per la raccolta dati e per la raccolta di materiale biologico, stoccaggio e trasporto secondo il protocollo dello studio, ed imput dei dati raccolti. Sarà inoltre necessaria la figura di uno statistico per l'analisi dei dati, in particolare l'analisi statistica seguirà l'analisi discriminante lineare di Fisher. Nella prima fase verrà studiata la distribuzione multivariata delle variabili. Successivamente sarà predisposto un modello probabilistico tenendo conto di variabili confondenti (età).Secondo obiettivo è l'analisi di valori prognostici dei markers nella popolazione in studio mediante modello di regressione di Cox's.

Faccio presente inoltre che nel progetto figurava la dr.ssa A. Seniori Costantini come coordinatore scientifico della Research dell'additional Unit ISPO dedicato alla parte epidemiologica e statistica, e che la sottoscritta, coinvolta comunque nel progetto, assumerà il ruolo di Coordinatore Scientifico e Responsabile del progetto.
Ricordo anche che le specifiche attività dell'ISPO in qualità di Additional Unit sono relative al terzo anno del progetto e che abbiamo ricevuto da parte dell'Università l'impegno del trasferimento dei fondi solo in data 6/02/2012 e che quindi l'importo a noi destinato verrà utilizzato interamente nel terzo anno.
Per quanto riguarda il progetto il PI Prof. Orlando ha già avuto parere positivo dal Comitato Etico di Careggi.

Il Responsabile del Progetto
Dr.ssa Lucia Miligi

Si approva
Il Referente Scientifico
Dr. Eugenio Paci

Sede legale:
ISPO Via Cosimo Il Vecchio 2 • 50139 Firenze
Telefono centralino 055 3269771

www.ispo.toscana.it

Ente costituito con LR Toscana n. 3 del 04.02.2008
CODICE FISCALE 94158910482
P. IVA 05872050488

ALLEGATO "C" ALLA DELIBERA D.G. N. 111 DEL 27/09/2012

ISTITUTO PER LO STUDIO E LA PREVENZIONE ONCOLOGICA

Servizio Sanitario della Toscana

PIANO ECONOMICO-FINANZIARIO
PROGETTI FINALIZZATI

Struttura organizzativa proponente:	S.C. Epidemiologia ambientale ed occupazionale		
Responsabile del progetto:	Dr.ssa Lucia Miligi		
Titolo del progetto:	Identification and clinical evaluation of genetic and epigenetic multiple markers in cell-free plasma DNA of melanoma patients		
Ente finanziatore:	Università degli Studi di Firenze - Dipartimento di Fisiopatologia Clinica		
Importo finanziamento:	€ 33.000,00		
Delibera numero:			
Codice Autorizzazione:	63/12		
Centro di Costo:	675		
Data inizio progetto:	15 aprile 2012		
Data conclusione progetto:	14 aprile 2013		
Modalità di pagamento:	70% inizio anno, 30% fine anno		
	ANNO 2012/13	**Totale**	**VOCE DI SPESA CORRISPONDENTE ENTE EROGATORE**
Beni di consumo:			
- cancelleria ed altri beni economali *(es. stampati, mouse, ...)*			
- farmaci			
- presidi (es. guanti, sonde,...)			
- diagnostici *(es.reagenti di laboratorio, test HPV, ...)*			
- acquisto libri e riviste (anche su supporto informatico; riviste on line)			
- altro (specificare)			
Beni di tipo strumentale:			
- attrezzature sanitarie			
- attrezzature informatiche e altro non sanitario *(es. computer, stampanti,... importi > 516,00 euro; per importi < 516,00 euro riferirsi a beni di consumo)*			
Beni immateriali:			
- software, opere di ingegno, brevetti			
Servizi:			
- Acquisto prestazioni sanitarie (es. prestazioni di laboratorio)			
- Acquisto prestazioni non sanitarie (es. servizio elabor.dati)			
- Spese per pubblicazioni			
- Spese per organizzazione convegni e congressi (es. cene, coffee break, ...)			
- Spese postali			
- Spese telefoniche			
Trasferimenti/ finanziamenti ad altri enti			
Personale	30.000,00	30.000,00	non-staff personnel
- collaborazioni, consulenze ed incarichi professionali	30.000,00	30.000,00	
- personale dipendente, tempo determinato			
- personale dipendente, tempo indeterminato			
Rimborsi			
- missioni/rimborsi spese collaborazioni, consulenze ed incarichi professionali			
- missioni/rimborsi spese dipendenti, tempo determinato			
- missioni/rimborso spese tempo indeterminato (incluso PI)			
Altro (specificare)			
Progetti del personale			
Spese generali di gestione (overheads)	3.000,00	3.000,00	overhead
Totale	33.000,00	33.000,00	

le celle in grigio si riferiscono a voci di costo non ammissibili in quanto non previste nel piano dell'Ente finanziatore

Firma Responsabile del progetto Lucia Miligi

Firenze, 11/9/12

Firma Resp. Struttura Org. Dr.ssa Chiara Neri
Direttore Sanitario ISPO