SENATO ACCADEMICO
Seduta del 8 luglio 2008

Sono presenti: il Rettore, Prof. Renato Guarini, Presidente ed i componenti del Senato Accademico: Prof. Carlo Angelici, Prof. Salvatore Dierna, Prof. Guido Martinelli, Prof. Attilio Celant, Prof. Fulco Lanchester, Prof.ssa Gabriella Salinetti, Prof.ssa Marta Fattori, Prof. Mario Morcellini, Prof. Gian Vittorio Caprara, Prof. Vincenzo Ziparo, Prof. Elvidio Lupia Palmieri, Prof. Fabrizio Vestroni, Prof. Lucio Barbera, Prof. Marcello Scalzo, Prof. Marco Merafina, Prof. Livio De Santoli, Prof. Raffaele Panella, Prof. Filippo Sabetta, Prof.ssa Rosanna Pettinelli, Prof. Luciano Zani, Prof. Mario Caravale (entra ore 16.20), Prof. Ernesto Chiacchierini, Prof.ssa Simona Pergolesi, Prof. Nino Dazzi, Prof.ssa Anna Maria Aglianò, Prof. Luca Tardella, Prof. Guido Valesini, Prof. Enrico Fiori, Prof. Alfredo Antonaci, Sig. Sandro Mauceri, Sig. Nicola Azzarito, Sig. Francesco Brancaccio, Sig. Luca Gentile, Sig.ra Marianna Massimiliani, Sig. Massimiliano Rizzo e il Dott. Carlo Musto D'Amore che assume le funzioni di Segretario.

Assistono i Presidi Proff.ri: Benedetto Todaro, Guido Pescosolido, Federico Masini, Luciano Benadusi, Stefano Puglisi Allegra, Luigi Frati, Attilio De Luca e Filippo Graziani e Mario Docci Presidente del Collegio dei Direttori di Dipartimento.

Assente giustificato: Prof. Domenico Misiti.

Assenti: Prof. Roberto Palumbo, Prof. Franco Chimenti, Prof. Aroldo Barbieri e il Sig. Livio Orsini.

.....o m i s s i s .....

BREVETTO ITALIANO IT1333549 DEL 4.5.2006 E PCT IT/03/000273 (EP APPLICATION 1501931) DEL 6.5.2003 DI PROPRIETÀ DELL'UNIVERSITA' DEGLI STUDI DI ROMA "LA SAPIENZA": CONTRATTO DI LICENZA E SPERIMENTAZIONE.

Il Presidente presenta per la discussione la seguente relazione predisposta dal Settore Trasferimento Tecnologico e Spin Off dell'Ufficio Valorizzazione Ricerca Scientifica e Innovazione.

Si ricorda che, in data 21.12.07, è stato sottoscritto, con la società Amsterdam Molecular Therapeutics B.V., il Term Sheet (approvato dal C.d.A. di questa Università nella seduta dell'11.12.07) per la concessione di una opzione sui diritti di proprietà intellettuale di cui in oggetto e finalizzato alla conclusione di un contratto di licenza e sperimentazione.

In merito si ritiene utile ricordare che l'Università è titolare al 100% in via originaria del Brevetto italiano IT1333549 del 4.5.06 e relativo PCT IT/03/000273. La relativa disciplina è regolata in base al Regolamento Brevetti di Ateneo approvato il 13.12.99 per le invenzioni sottoposte a tutela prima dell'entrata in vigore della Legge n. 383/2001 ("Tremonti bis", art.7).

A seguito della volontà della A.M.T., espressa formalmente in data 26.2.08, di addivenire alla stipula del definitivo contratto, è stato messo a punto, sulla base dei termini e delle condizioni già fissate nel Term Sheet, il definitivo contratto di licenza, immediatamente sottoscritto dal Rettore, in data 9.5.08, per motivi di urgenza correlati alla necessità (per A.M.T.) di avviare subito i protocolli di attività prevista.

Il contratto costituisce un "unicum" negoziale di natura mista in quanto disciplinante: a) la concessione in licenza dei diritti di sfruttamento industriale sulla proprietà intellettuale; b) lo sviluppo e la sperimentazione del trovato per la messa a punto di una terapia potenzialmente efficace contro la malattia di Duchenne: c) l'attività di ricerca su nuove mutazioni genetiche della medesima malattia.

Esso prevede i seguenti obblighi e movimenti finanziari:

- pagamento, da parte di A.M.T. di una lump-sum iniziale pari a € 300.000,00 (trecentomila) al momento della sottoscrizione;
- successivi pagamenti, subordinati alla concessione del brevetto nei Paesi europei che rappresentano la maggioranza della popolazione della U.E. o comunque nei paesi scelti da A.M.T., e corrisposti a fronte di specifiche fasi di sperimentazione documentate dai rispettivi stati di avanzamento, così articolate:

  step 1: € 400.000,00 : prima proposta IND del primo skipping dell'esone 51;

  step 2: € 400.000,00: prima dose al primo paziente per la prova clinica pilota;

  step 3: € 400.000,00: in caso di successo del punto 1 e 2, prima registrazione pilota;

  step 4: € 2 milioni - approvazione da parte dell'EMEA;

  step 5 € 1,5 milioni - approvazione da parte della FDA;
- pagamento da parte di A.M.T. di royalties pari al 5% del fatturato netto generato dalla vendita dei prodotti per la terapia contro la malattia di Duchenne, nei soli Paesi in cui il brevetto è stato rilasciato; nei Paesi in cui il rilascio non è avvenuto la percentuale di royalties si ridurrà del 50%;

- accollo, da parte di A.M.T., dei costi brevettuali relativi ad esame, deposito, nazionalizzazione e mantenimento della domanda di brevetto dal momento della sottoscrizione;
- pagamento da parte di A.M.T di una ulteriore somma pari ad € 120.000,00 (centoventimila) annuali, per un periodo minimo di 3 anni, al fine di proseguire l'attività di ricerca relativa alla terapia per la malattia di Duchenne. Di supporto all'attività di ricerca è prevista la creazione di un Comitato Scientifico, composto da membri di entrambe le Parti, con compiti di valutazione dei risultati raggiunti.

La *lump sum* iniziale è destinata alla copertura della concessione in licenza esclusiva del brevetto e al trasferimento del know-how (definito nel contratto come "University Know-how and University Material") da mettere a punto (e trasferire alla A.M.T.) da parte del gruppo di ricerca della prof.ssa Bozzoni (coordinatrice del progetto).

Si prospetta quindi la necessità, evidenziata dalla stessa prof.ssa Bozzoni, di disporre delle risorse finanziarie necessarie, quantificate in un importo complessivo pari a € 90.000,00, per ottemperare all'obbligo sopra citato e completare la messa a punto della molecola da testare (passaggio inderogabile per l'accesso all'operazione contrattuale e all'adempimento degli obblighi con essa assunti).

A tal proposito, la Commissione Tecnica Brevetti, nella seduta del 7.3.08, dopo un approfondito esame teso a considerare sia gli aspetti di alto contenuto morale ed etico sottesi all'operazione negoziale (e relativi alla possibilità della messa a punto di una terapia genica efficace contro la malattia di Duchenne), sia le opportunità contrattuali insite nella stessa, ha valutato l'ipotesi di reinvestire una quota parte degli utili netti dell'Università nel completamento e la finalizzazione della sperimentazione.

Quanto sopra in conformità alle disposizioni di cui all'art. 7 del Regolamento Brevetti del 13.12.99, ai sensi del quale gli utili netti derivanti dai contratti di licenza e simili, che confluiscono nel Fondo Brevetti, sono destinati al finanziamento di attività di ricerca e brevettazione.

Tale sistema consentirebbe la disponibilità della somma necessaria, attraverso un meccanismo di compartecipazione alle spese tra Dipartimento di Genetica e Biologia Molecolare e Amministrazione centrale.

Al termine della seduta, la C.T.B. si è infatti espressa nei termini seguenti: *"la Commissione al termine della discussione, esprime parere favorevole sulla bozza di contratto di licenza e sperimentazione con la Società AMT e sugli aspetti economico-finanziari dello stesso, e ferma restando l'esigenza, sancita dal Regolamento, di garantire il ripianamento dei costi diretti e indiretti per gli oneri di brevettazione, approva all'unanimità la proposta di garantire, attraverso la messa a disposizione della quota di utili del Dipartimento e il re-investimento di una quota di utili netti spettanti all'Università, la disponibilità complessiva di € 90.000,00 per la produzione della molecola da fornire alla Società AMT attraverso il completamento del protocollo di sperimentazione"*.

In considerazione di ciò, si è ipotizzato che il corrispettivo iniziale di € 300.000,00, debba in primo luogo essere destinato a ripianare i costi di brevettazione sostenuti dall'Amministrazione e ammontanti ad oggi a € 19.596,30; la somma residua verrà ripartita:

- per il 30% (pari a € 84.121,11) agli autori dell'invenzione;

- per il 20% (pari a € 56.080,74) alla struttura di appartenenza (nel caso in esame il Dipartimento di Genetica e Biologia Molecolare “C. Darwin”);
- per il 50% (pari a € 140.921,85) all’Amministrazione Centrale.

Seguendo la proposta avanzata dalla Commissione Tecnica Brevetti, una quota parte degli utili spettanti all’Università corrispondente a € 33.919,26, dovrebbero essere trasferiti al Dipartimento di Genetica e Biologia Molecolare “C. Darwin” per consentire, assieme alla quota di utile del Dipartimento medesimo (€ 56.080,74), la disponibilità della somma necessaria per la messa a punto della molecola da testare.

I successivi pagamenti previsti negli *steps* dal n. 1 al n. 5 di cui sopra, sono destinati a finanziare la seconda fase di sviluppo e sperimentazione che verrà condotta dal gruppo di ricerca coordinato dalla prof.ssa Bozzoni, il quale affiancherà A.M.T. in collaborazione anche con la “Parent Project Onlus” (associazione promossa dai genitori con figli affetti da Distrofia muscolare Duchenne e Becker). Tale fase si concretizzerà in un’attività di studio e monitoraggio molecolare, dal momento della sperimentazione preclinica e clinica fino a quello dell’immissione sul mercato del farmaco con azione terapeutica efficace contro la Distrofia Muscolare di Duchenne (DMD).

I relativi finanziamenti, introitati dall’Università (quale soggetto garante della conclusione e della regolare esecuzione della complessiva operazione), saranno successivamente trasferiti al Dipartimento di Genetica e Biologia Molecolare.

L’ulteriore finanziamento previsto dal contratto, pari a € 360.000,00 suddivisi in tre rate annuali, è destinato a finanziare la ricerca di base su nuove mutazioni genetiche riguardanti la malattia di Duchenne; a tal fine, anche tali somme, una volta riscosse dall’Università, verranno trasferite al Dipartimento di Genetica e Biologia Molecolare “C. Darwin” e vincolate al finanziamento di tale specifico progetto, sulla base di uno specifico accordo interno.

Stante quanto sopra, si sottopone a questo Consesso la proposta formulata dalla Commissione Tecnica Brevetti, di reinvestire una quota parte di utili spettanti all’Università, per consentire al gruppo di ricerca coordinato dalla prof.ssa Bozzoni la messa a punto della molecola (da trasferire poi ad A.M.T.) necessaria per l’avvio della sperimentazione.

In data 6.5.08 è stato richiesto il parere del Collegio dei Sindaci, sulle variazioni di bilancio che si rendono necessarie per introitare ed allocare i finanziamenti; le medesime variazioni, sulle quali in data 4.6.08 con verbale n. 510 il Collegio medesimo ha espresso parere favorevole, sono state approvate dal C.d.A. di questa Università nella seduta del 24.6.08.

Allegati parte integrante:

1) contratto di licenza e sperimentazione di A.M.T. ;
2) verbale della Commissione Tecnica Brevetti del 7.3.08;
3) dichiarazione di impegno tra Amministrazione Centrale e Dipartimento di Genetica e Biologia Molecolare “C. Darwin”
4) progetto di ricerca sulla malattia di Duchenne (summary);

Allegati in visione:

1) decreto Rettorale n. 230 del 9.5.2008
2) delibera del C.d.A. dell’11.12.07 di approvazione del Term Sheet;
3) regolamento brevetti del 13.12.99.

Il Presidente pone in votazione la proposta di delibera.

IL SENATO ACCADEMICO

LETTA la relazione predisposta dal Settore Trasferimento Tecnologico e Spin Off dell'Ufficio Valorizzazione Ricerca Scientifica e Innovazione;

VISTO il Regolamento Brevetti del 13.12.99 in vigore anteriormente alla riforma attuata dalla Legge 383/01 e applicabile alla fattispecie richiamata nella relazione;

VISTO il brevetto italiano n. 1333549 del 4.5.06, dal titolo "Molecole chimeriche di SnRNA con sequenze antisenso per le giunzioni di splicing del gene della distrofina e applicazioni terapeutiche" – inventori: prof.ssa Irene Bozzoni e dott.ssa Fernanda Gabriella De Angelis;" – esteso all'estero con PCT/IT2003/000273 del 6.5.03 – titolarità in via originaria: La Sapienza 100%;

VISTO il Term Sheet approvato dal C.d.A. in data 11.12.07 e sottoscritto con la società Amsterdam Molecular Therapeutics B.V. (A.M.T.) in data 21.12.07;

VISTO il contratto di licenza e sperimentazione sottoscritto con la A.M.T. in data 9.5.08, relativo alla concessione in licenza esclusiva mondiale dei relativi diritti di sfruttamento sui brevetti di cui sopra e al finanziamento della fase di sviluppo e sperimentazione;

CONSIDERATI i presupposti di necessità che hanno determinato l'esigenza di sottoscrivere d'urgenza il contratto nei termini previsti per il rispetto delle *due-diligence* reciprocamente stabilite e per il tempestivo avvio del protocollo di sperimentazione;

CONSIDERATI gli aspetti di alto contenuto morale ed etico sottesi a tale operazione negoziale e relativi alla possibilità di giungere alla messa a punto di una terapia genica efficace contro la malattia di Duchenne;

PRESO ATTO del parere favorevole reso al riguardo dalla Commissione Tecnica Brevetti, nella riunione del 7.3.08 e della proposta della medesima inerente il reinvestimento di una quota parte di utili spettanti all'Università per la messa a punto della molecola da testare e trasferire ad A.M.T.;

con voto unanime

ESPRIME PARERE FAVOREVOLE

1) in merito agli aspetti di carattere scientifico, istituzionale, etico e morale del progetto di collaborazione;
2) in merito alla proposta di reinvestimento di una quota parte degli utili spettanti all'Università, per un importo di € 33.919,26 per consentire la messa a punto della molecola da testare, quale

113

passaggio necessario volto ad adempiere agli obblighi assunti con l'operazione contrattuale di cui in oggetto;
3) in merito alla dichiarazione di impegno concordata con il Dipartimento di Genetica e Biologia Molecolare "C. Darwin" inerente la compartecipazione alle spese di sperimentazione di cui al punto precedente per i rispettivi importi ivi indicati.

Letto ed approvato seduta stante per la sola parte dispositiva.

IL SEGRETARIO
Carlo Musto D'Amore

IL PRESIDENTE
Renato Guarini

THIS LICENSE AND SPONSORED RESEARCH AGREEMENT ("**Agreement**") is made the day of ("**Effective Date**")

BETWEEN

(1) **AMSTERDAM MOLECULAR THERAPEUTICS B.V.,** a closed limited liability company organized and existing under the laws of the Netherlands, with registered offices at Meibergdreef 61, 1105 BA Amsterdam, the Netherlands ("**AMT**"); and

(2) **LA SAPIENZA UNIVERSITY**, Rome, whose principal address is P.le Aldo Moro, 5 - 00185, Rome, Italy, fiscal code 80209930587, VAT No. 02133771002 ("**University**"),

(each a "**Party**" and together the "**Parties**").

RECITALS

A. AMT is a company engaged in the research, development, manufacture and commercialisation of certain therapeutic and prophylactic products and technologies particularly in the field of gene therapy.

B. The University is a leading academic institution at which Dr Irene Bozzoni ("**Dr Bozzoni**") and Dr Fernanda Gabriella De Angelis ("**Dr De Angelis**") work (Drs Bozzoni and De Angelis together being (the **"Researchers"**)) in the department of genetics and molecular biology (the "**Department**") in their laboratory (**"Researchers Laboratory"**).

C. In 2002 Dr Bozzoni and Dr De Angelis conceived an invention concerning a gene transcribing a modified small nuclear RNA antisense sequence that is involved in the splicing of dystrophin pre-mRNA. A patent application was filed in respect of this invention which has been assigned to and is owned by the University.

D. The University wishes to grant AMT a license under the Patent Application (defined below) to develop a gene therapy product to treat Duchennes Muscular Dystrophy under the terms and conditions set forth in this Agreement.

E. Dr Bozzoni and Dr De Angelis have obtained a grant from Telethon, an Italian charity, (Grant No. GGP07049, as may be renewed or as further grants may be made to the Department of the University from time to time) ("**Telethon Grant**"), under the terms of

which all intellectual property rights in the results of the work carried out pursuant to the Telethon Grant are vested in the University.

F. AMT also wishes to sponsor additional research work in the Researchers Laboratory for a minimum of three years in the general exon skipping field. The University wishes the Researchers to carry out this research in the Researchers Laboratory and to assign to AMT ownership of all intellectual property rights in the results of such research sponsored by AMT and to grant to AMT an option to license all intellectual property rights in the results of the research carried out pursuant Telethon grant monies.

THE PARTIES HAVE NOW AGREED AS FOLLOWS:

1. **DEFINITIONS**

In this Agreement and in the Schedules to this Agreement the following words and phrases shall have the following meanings, unless the context requires otherwise:

1.1 "**Affiliate**" means any company, partnership or other business entity which Controls, is Controlled by or is under common Control with either Party. For the purposes of this definition "Control" means any of the following: (i) the possession, directly or indirectly, of the power to direct the management or policies of an entity, whether through ownership of voting securities, by contract or otherwise; (ii) ownership of fifty percent (50%) or more of the voting securities entitled to vote for the election of directors in the case of a corporation, or of fifty percent (50%) or more of the equity interest in the case of any other type of legal entity; (iii) status as a general partner in any partnership, or any other arrangement whereby a Party controls or has the right to control the board of directors or equivalent governing body of a corporation or other entity.

1.2 "**AMT IP**" means AMT Know How, AMT Materials and AMT Patent Rights.

1.3 "**AMT Know How**" means any Know How (i) Controlled by AMT at the Effective Date and relating to a Vector or the manufacture of Vectors or (ii) generated by or on behalf of AMT during the term of this Agreement and relating to the Product, a Vector or the manufacture of Product and/or Vectors.

1.4 "**AMT Materials**" means any Materials (i) Controlled by AMT at the Effective Date comprising a Vector or used in the manufacture of Vectors or (ii) generated by or on behalf of AMT during the term of this Agreement comprising the Product, a Vector or which are used in the manufacture of Product and/or Vectors.

1.5 "**AMT Patent Rights**" means any and all Patent Rights Controlled by AMT at any time Covering any aspect of the AMT Know How or Covering the AMT Materials.

1.6 "**Commercialisation**", "**Commercialising**", or "**Commercialise**" means all activities relating to the import, advertising, promotion and other marketing, pricing and reimbursement, detailing, distribution, storage, handling, offering for sale and selling, customer service and support or post Regulatory Approval regulatory activities in relation to Product.

1.7 "**Commercially Reasonable Efforts**" in respect of AMT means efforts and resources commonly used by biotechnology companies of a similar size to AMT based on funds raised to Develop and Commercialise a product owned by such a company or to which it has rights, which product is at a similar stage in its development or product life and is of similar market potential to Product and taking into account the patent and other proprietary position of the product.

1.8 **"Competent Authority"** means any national or local agency, authority, department, inspectorate, minister, ministry official, parliament or public or statutory person (whether autonomous or not) of any government of any country having jurisdiction over either any of the activities contemplated by this Agreement or over the Parties.

1.9 "**Confidential Information**" means the following, subject to the exceptions set forth in Section 11.1: (i) the terms and conditions of this Agreement, for which each Party will be considered a Disclosing Party and a Receiving Party; (ii) Know How within AMT Know How and University Know How as the case may be for which the Party disclosing such Know How will be considered the Disclosing Party and the other Party shall be the Receiving Party; (iii) Know How within Research IP in relation to which AMT will be considered the Disclosing Party regardless of the fact that AMT may receive the same from the University, and (iv) Know How within Optioned IP in relation to which the University will be considered Disclosing Party and (v) any other non-public information, whether or not patentable, disclosed or provided by one Party

to the other Party in connection with this Agreement, including, without limitation, information regarding such Party's strategy, business plans, objectives, research, technology, products, business affairs or finances including any non-public data relating to commercialisation of any product and other information of the type that is customarily considered to be confidential information by parties engaged in activities that are substantially similar to the activities being engaged in by the Parties under this Agreement, for which the Party making such disclosure will be considered the Disclosing Party and the receiver will be the Receiving Party.

1.10 "**Control**" (including variations such as "**Controlled**") means with respect to any Know How, or Patent Rights possession of the right, whether directly or indirectly, and whether by ownership, licence or otherwise, to assign, or grant a license, sub-license or other right to or under, such Know How or Patent Right without violating the terms of any agreement or other arrangement with any third party.

1.11 "**Cover**" (including the variations such as "Covered", "Coverage" or "Covering"), with respect to a Patent Right, means that the making, using or other commercialisation of a given product in a commercialised form would infringe a Valid Claim of such Patent Right in the country where such activity occurs in the absence of a license under such Patent Right, and with respect to Know-How, shall means that the making, using or other commercialisation of a given product in a commercialisable form would require use of such Know How.

1.12 **"Development"** (and the corresponding verb "to Develop")" means all pre-regulatory approval development and regulatory activities regarding a product in a country conducted with the aim of obtaining such regulatory approval including:

(a) studies on the toxicological, pharmacological, metabolical or clinical aspects of a product conducted internally or by individual investigators or consultants; and

(b) process development, validation, stability, test method development, process improvement, scale-up and recovery, the manufacture of clinical supplies of products, (including failed batches) and the manufacture of qualification lots; and

(c) preparing, submitting, reviewing or developing data or information for the purpose of submission to a regulatory authority to obtain, maintain and/or expand the manufacturing approval and/or regulatory approval of products including data management, statistical designs and studies, document preparation, and other administration and,

(d) all post regulatory approval regulatory activities including Phase IV Clinical Trials and adverse event reporting in relation to products.

1.13 "**Disclosing Party**" means the Party which transfers Materials or discloses Know How to the other Party.

1.14 "**Documents**" means analyses, books, charts, CD-ROM, comments, computations, computer information storage means, written material and any other media on which Know How can be permanently stored, computer programs and documents thereof, discs, diskettes, files, graphs, ledgers, notebooks, paper, photographs, records, recordings, reports, research notes and tapes.

1.15 **"IND"** means an investigational new drug application filed with, and accepted by, the FDA prior to beginning clinical trials in humans in the USA, or any comparable application to and acceptance by the Regulatory Authority of a country or group of countries other than the USA thereto including but not limited to the EMEA prior to beginning clinical trials in humans in that country or in that group of countries.

1.16 "**Insolvency Event**" means in relation to a Party, means any one of the following:

(a) a resolution shall have been passed by that Party's directors or by its shareholders to request a court to open insolvency proceedings as defined in Article 2(a) of the European Insolvency Regulation; or

(b) a request to open such insolvency proceedings shall have been submitted by that Party to a court; or another person or entity who has the power to request the opening of insolvency proceedings as defined in Article 2(a) of the European Insolvency Regulation shall have submitted such request; or insolvency proceedings as defined in Article

2(a) of the European Insolvency Regulation have been opened with respect to that Party; or

(c) a request has been filed for suspension of debts of a Party; or

(d) a Party, as a consequence of financial difficulties, makes any voluntary arrangement with its creditors outside a bankruptcy, suspension of payments or any other similar regulation; or

(e) an order is made or a resolution passed for the winding-up of a Party.

1.17 "**Know How**" means confidential, non-public unpatented technical and other information including information comprising or relating to concepts, discoveries, data, designs, formulae, ideas, information relating to Materials, inventions, methods, models, assays, research plans, procedures, designs for experiments and tests and results of experimentation and testing (including results of research or development) processes (including manufacturing processes, specifications and techniques), laboratory records, chemical, pharmacological, toxicological, clinical, analytical and quality data, trial data, case report forms, data analyses, reports, manufacturing data or summaries and information contained in submissions to an information from ethical committees and regulatory authorities. Know How includes Documents containing Know How. Know How includes any rights including copyright, database or design rights protecting such Know How.

1.18 **"Laboratory"** means the laboratory in which Prof. Bozzoni and Dr. De Angelis carry on the project on the Gene Therapy of Duchenne Muscular Dystrophy.

1.19 "**Legal Requirement**" means any present or future law, regulation, directive, instruction, direction or rule of any competent authority or regulatory authority including any amendment, extension or replacement thereof which is from time to time in force including, but without limitation, those relating to the safety of Products.

1.20 "**Major Markets**" means USA, EU and Japan (and each a "**Major Market**").

1.21 "**Materials**" means any:

(a) organic or inorganic compound;

(b) nucleotide or nucleotide sequence including DNA and RNA sequence;

(c) gene;

(d) vector or construct including plasmids, phages or viruses;

(e) host organism including bacteria, fungi, yeast, algae, protozoa and hybridoma;

(f) eukaryotic or prokaryotic cell line or expression system or any development strain or product of that cell line or expression system;

(g) protein including any peptide or amino acid sequence, enzyme, antibody or protein conferring target properties and any fragment of a protein, peptide, enzyme or antibody;

(h) drug or pro-drug;

(i) assay or reagent;

(j) or any other genetic or biologic material or micro-organism.

1.22 "**Net Sales**" - the gross amount invoiced for sales of Product, in arm's length sales by AMT, or its Affiliates to third parties, less the following deductions from such gross amounts which are actually incurred, allowed, accrued or specifically allocated:

(a) normal and customary trade, cash and quantity discounts actually given, credits, price adjustments or allowances for damaged Products, returns or rejections of Products;

(b) chargeback payments and rebates (or the equivalent thereof) for Product granted on a customary trade basis to group purchasing organisations, managed health care organisations or to federal, state/provincial, local and other governments, including their agencies, or to trade customers;

(c) reasonable and customary freight, shipping insurance and other transportation expenses directly related to the sale of Product (if

actually borne by AMT or its Affiliates without reimbursement from any third party);

(d) required distribution commissions/fees payable to any third party providing distribution services to AMT;

(e) sales, value-added, excise taxes, tariffs and duties, and other taxes and government charges directly related to the sale, to the extent that such items are included in the gross invoice price of Product and are actually borne by AMT, its Affiliates, without reimbursement from any third party (but not including taxes assessed against the income derived from such sale); and

(f) actual uncollectible amounts for Product where collectibility is determined in accordance with IFRS consistently applied to all AMT products.

The transfer of ownership of Product by AMT or one of its Affiliates to another Affiliate shall not be considered a sale.

It is in any event understood that in the case of any sale which is not invoiced or is delivered before invoice, Net Sales shall be calculated at the time of shipment or when the Product is paid for, if paid for before shipment or invoice, provided that the relevant gross amount shall be equal to the amount actually invoiced.

Upon the sale or other disposal of Product other than in a transaction generating revenues from or based on a sales price for Product which sales price is either customary or would be reasonably expected in the Territory, such sale, disposal or use shall be deemed to constitute a sale with the consideration for the sale being the consideration for the relevant transaction and constituting Net Sales hereunder or if the consideration is not a monetary amount a sale shall be deemed to have occurred for a price assessed on the value of whatever consideration has been provided in exchange for the supply. Disposal of Product for or use of Product in clinical trials or as free samples shall not give rise to any deemed sale under this Section. For clarity, there shall be no limit on the quantity of Product which may be used in clinical trials.

Such amounts shall be determined from the books and records of AMT maintained in accordance with IFRS, consistently applied.

1.23 "**Optioned IP**" means any and all Know How and Patent Rights arising out of University research in Drs Bozzoni and De Angelis' laboratory that was funded in whole or in part by the Telethon Grant.

1.24 "**Patent Application**" means the granted Italian patent number 1333549 and corresponding European patent application EP 1501931.

1.25 "**Patent Rights**" means:

(a) all national, regional and international patents and patent applications, including provisional patent applications,

(b) all patent applications filed either from such patents, patent applications or provisional applications or from an application claiming priority from any of these, including divisionals, continuations, continuations-in-part, provisionals, converted provisionals, and continued prosecution applications,

(c) any and all patents that have issued or in the future issue from the foregoing patent applications (a) and (b), including author certificates, inventor certificates, utility models, petty patents and design patents and certificates of invention,

(d) any and all extensions or restorations by existing or future extension or restoration mechanisms, including revalidations, reissues, re-examinations and extensions (including any supplementary protection certificates and the like) of the foregoing patents or patent applications (a), (b) and (c), and

(e) any similar rights, including so-called pipeline protection, or any importation, revalidation, confirmation or introduction patent or registration patent or patent of additions to any such foregoing patent applications and patents.

1.26 "**Product"** means a product for the therapy or prophylaxis of Duchennes Muscular Dystrophy containing Transgene.

1.27 "**Quarter"** means each period of three months ending on 31 March, 30 June, 30 September or 31 December and "Quarterly" shall be construed accordingly.

1.28 "**Receiving Party**" means the Party which receives Materials or Know How from the other Party.

1.29 "**Regulatory Approval**" means any approval required from a Regulatory Authority to market and sell Product in any country of the Territory but excluding any pricing or reimbursement approval required or commercially desirable.

1.30 "**Regulatory Authority**" means any national, supranational, regional, state or local regulatory agency, department, bureau, commission, council or other governmental entity including the FDA in any country involved in the granting or receipt as the case may be of Regulatory Approvals.

1.31 "**Research IP**" means Research Know How, Research Materials and Research Patent Rights.

1.32 "**Research Know How**" means any Know How generated by or on behalf of the University in the conduct of the Research Project in the Researchers Laboratory.

1.33 "**Research Materials**" means any Materials generated by or on behalf of the University in the conduct of the Research Project in the Researchers Laboratory.

1.34 "**Research Patent Rights**" means any and all Patent Rights at any time Covering any aspect of the Research Know How or Covering the Research Materials.

1.35 "**Research Project**" means the research project to be conducted by the University sponsored by AMT in the Researchers Laboratory as set out at Schedule 1.

1.36 "**Research Term**" The time period during which the Research Project is being conducted by the University in the Researchers Laboratory with the initial term of the Research Project being a period of three (3) years from the Effective Date ("**Initial Research Term**") including any extensions agreed between the Parties.

1.37 **"Semester"** means each period of six (6) months ending on 15 June, 15 December.

1.38 **"Territory"** means worldwide.

1.39 "**Transgene**" means the nucleotide sequence construct having the sequence(s) and otherwise as claimed in the Patent Application or subsequent Patent Rights arising therefrom.

1.40 "**University IP**" means University Know How, University Materials and University Patent Rights.

1.41 "**University Know How**" means any Know How (i) Controlled by the University at the Effective Date and relating to the Transgene or (ii) generated by or on behalf of the University during the term of this Agreement outside the Research Project and the Telethon Grant and relating to the Transgene.

1.42 "**University Materials**" means any Materials (i) Controlled by the University at the Effective Date comprising the Transgene or (ii) generated by or on behalf of the University during the term of this Agreement outside the Research Project and the Telethon Grant.

1.43 "**University Patent Rights**" means (i) the Patent Application and (ii) any and all Patent Rights Controlled by the University at any time (including pursuant to the terms of this Agreement) Covering any aspect of the University Know How or Covering the University Materials.

1.44 "**Valid Claim**" means a claim of an issued and unexpired patent included within Patent Rights, which has not been held permanently revoked, unenforceable or invalid by a decision of a court or other governmental agency of competent jurisdiction, unappealable or un-appealed within the time allowed for appeal, and which has not been admitted to be invalid or unenforceable through reissue or disclaimer or otherwise.

1.45 "**Vector**" means any vector used in the Development of Product by AMT including but not limited to (i) the AAV protein capsid that encases a Transgene; (ii) any expression cassette and (iii) any components of (i) or (ii) but always excluding a Transgene itself.

2. **LICENCE**

The University hereby grants to AMT and its Affiliates under the University IP the exclusive worldwide sub-licensable right to research, have researched, Develop, have Developed make, have made, use, Commercialise and have Commercialised Product in the Territory.

3. **GOVERNANCE**

3.1 With effect from the Effective Date the Parties shall establish and run a liaison committee ("**LC**") as the principal forum for liaison and communication between the Parties as follows:

3.1.1 The LC shall consist of six (6) persons ("**LC Members**"), three (3) members to be designated by the University and three (3) members to be designated by AMT. Dr Bozzoni shall be one of the University's LC Members. Each Party shall notify the other Party of the member(s) designated by such Party, in writing, within thirty (30) days after the Effective Date. AMT and the University shall each notify the other of any change in the identities of their LC Members. Both sides shall use reasonable efforts to keep an appropriate level of continuity in representation and to ensure that LC Members have familiarity and appropriate knowledge of the activities to be discussed by the LC. LC Members may be represented at any meeting by another person designated in writing by the absent LC Member. The chairperson of the LC shall be from AMT; and

3.1.2 no later than seven (7) days prior to each meeting of the LC, each Party will provide the other with written copies of all materials that Party intends to present at the LC meeting;

3.1.3 either party may request the attendance of other specialists at LC meetings, for example its nominated patent attorney, but only as a non-member and subject to a confidentiality obligation as provided in Section 11.

3.2 The LC:

3.2.1 shall hold meetings in person or by teleconference or video-conference as frequently as the members of the LC may agree shall be necessary but in any event no less frequently than four (4) times each calendar year. Meetings may be requested by either Party and dates of meetings shall be agreed by the Parties not less than thirty (30) days beforehand; responsibility for arranging the meetings, including, at least, providing written notice and an agenda, shall be the responsibility of the Chairperson. The first meeting of the LC will take place as soon as practicable after the Effective Date, but in no event later than thirty (30) days after the Effective Date, and will be organized by AMT;

3.2.2 shall receive, no later than seven (7) days prior to each meeting, a written progress report from AMT in connection with the Development of Product including but not limited to the Development Reports if requested under Section 4.3. LC Members shall have an opportunity to comment upon such progress reports from AMT and, if AMT or any sub-licensee experiences technical problems in connection with the Development of Product, shall seek to resolve such technical problems;

3.2.3 shall receive from the University a plan of the activity to be carried out by the University during the following calendar year of the Research Term as a proposed update to the Research Project (see Section 7.4 below), and Semester Research Reports (see Section 7.5 below) and have an opportunity to comment upon the same;

3.2.4 LC minutes shall be drafted by each Party in turn no later than five (5) business days after the corresponding meeting. The non-drafting Party shall approve the draft minutes provided by the other Party within five (5) Business Days after notification. In case no express disapproval is granted by the non-drafting Party within the ten (10) days following notification, it shall be understood that the drafted minutes have been approved;

3.2.5 In the event that AMT sublicenses substantially all of its rights hereunder to a Third Party Licensee, the LC shall remain in place as an Information Review Committee (IRC) and shall meet at least once a year on written notification by either party. Updated information on the Development of Product shall be

brought to the attention of IRC by AMT representatives. The IRC shall remain the forum where LC Members shall be informed and discuss as regards the matters included in the LC's competence under this Agreement. In case of termination of such sublicense agreement the LC shall be immediately re-established in lieu of the IRC.

3.2.6 shall perform such other functions and responsibilities as are given to it under the express provisions of this Agreement but shall have no authority to amend any terms of this Agreement.

4. DEVELOPMENT BY AMT

4.1 On the Effective Date the University shall transfer to AMT the University Know How and the University Materials.

4.2 AMT shall use its Commercially Reasonable Efforts to carry out Development of the Product (including related manufacture) in accordance with all Legal Requirements until Regulatory Approval is achieved in any one of the Major Markets (which may entail the conduct of Development by itself, its collaborators or licensees). This shall be at the cost and expense of AMT, its collaborators or licensees.

4.3 In each calendar year during which Development on the Product is being conducted, within thirty (30) days after the end of each such calendar year, AMT shall prepare a written report regarding the Development Project and provide the same to the LC (together, the "**Development Report**"). The Development Report shall summarise the results generated as part of Development and not previously reported to the LC.

4.4 As and when AMT needs to carry out a clinical trial as part of the Development it shall make reasonable efforts to involve "Parent Project Onlus" both for the identification and involvement of patients and for organisational support where necessary in order to assure an Italian participation in such trial.

5. COMMERCIALISATION

5.1 AMT shall use Commercially Reasonable Efforts to Commercialise Product (including related manufacture and supplies) in any one of the Major Markets

(whether by itself, licensees, distributors or otherwise) in accordance with all Legal Requirements.

6. FINANCIALS

Signature fee

6.1 AMT will pay to the University an upfront fee of three hundred thousand Euro (€300,000) within ten (10) days of the Effective Date following receipt by AMT of an invoice therefor from the University.

Milestone payments

6.2 AMT shall pay the University the following milestone payments in Euro for the Product on the occurrence of the following milestone events provided that the Patent Application has proceeded to grant in the countries of the European Union comprising the majority of the population of the European Union, or has proceeded to grant in those countries where an application is requested by AMT if the populations in those countries do not in aggregate constitute the majority of the population of the European Union. If a milestone event occurs prior to the Patent Application proceeding to grant in the countries of the European Union comprising the majority of the population of the European Union, or proceeding to grant in those countries where an application is requested by AMT if the populations in those countries do not in aggregate constitute the majority of the population of the European Union, AMT shall pay the milestone payment upon the Patent Application so proceeding to grant.

Milestone Event	**Milestone Payment in Euro**
Submission of the first IND filing for a therapy for Duchennes disease with the EMEA or a similar filing with any competent authority in the USA in respect of the first exon 51 skipping product.	€400,000
First dosing of the first patient in the first pivotal registration trial in respect of the first exon 51 skipping product for a therapy for Duchennes	€400,000

Milestone Event	Milestone Payment in Euro
disease.	
Successful completion (determined by the fact that the primary and secondary end points of the trial are met) of the first pivotal registration trial in respect of the first exon 51 skipping product for a therapy for Duchennes disease.	€400,000
Grant of Regulatory Approval by the EMEA in respect of the first exon 51 skipping product for the therapy of Duchennes disease	€2,000,000
Grant of Regulatory Approval by the FDA in respect of the first exon 51 skipping product for the therapy of Duchennes disease	€1,500,000

6.3 Each of the milestone payments the subject of Section 6.2, shall only be payable by AMT upon the first occurrence of the applicable event whenever it occurs. Such milestone payments are non-refundable in any circumstances whatsoever and are not creditable against the royalties due under Section 6.5.

6.4 AMT shall report the occurrence of each milestone event under Section 6.2 to the University within thirty (30) days of its occurrence and at the same time shall make the milestone payment to the University for which Section 6.2 provides.

Royalty Payments

6.5 AMT shall pay to the University a royalty of:

6.5.1 five percent (5%) Net Sales on all Products on a Product-by-Product and country-by-country basis for so long as there are Valid Claims of Patent Application Covering Product in such country of sale; or

6.5.2 in countries where there are no Valid Claims of Patent Application Covering Product but the Product being sold there has utilised University Know How

royalties shall be payable by AMT at fifty per cent (50%) of the applicable royalty rate shown in Section 6.5.1 for a period of ten (10) years post-launch of Product.

For clarity, if neither of these criteria apply royalties shall not be payable in such country.

6.6 All sums due to the University under Section 6.5 shall be calculated and payable on a Quarterly basis, and shall be paid in Euro within forty five (45) days following the end of each calendar Quarter. Each such payment shall be accompanied by a written report indicating the amount of Net Sales in the Territory during such calendar Quarter, including quantity and price of Product sold, the currency conversion rates used, if any, and a calculation of the sums due.

6.7 Whenever for the purpose of calculating the payments due under Section 6.5 conversion from any foreign currency shall be required, such conversion shall be made as follows. The relevant amount in the foreign currency/ies shall be converted into Euro at the time payment is due using the average monthly rate of exchange for such currency/ies published in the Financial Times in accordance with AMT's then current standard practices.

6.8 If AMT becomes obliged by law to make a deduction or withholding in respect of tax from any amount payable under this Agreement it shall make this deduction.

6.9 AMT and its Affiliates shall keep and AMT shall require its licensees to keep, full, true and accurate records and books of account containing all particulars that may be necessary for the purpose of calculating all payments payable to the University for a minimum period of six (6) years. Upon timely request by the University, the University shall have the right to instruct an independent, internationally recognized, accounting firm acceptable to both the Parties to perform an audit of AMT and its Affiliates (including records of royalty accounting received from their Licensees, if any) conducted so far as appropriate in accordance with IFRS, as is reasonably necessary to enable such accounting firm to certify to the University whether the Net Sales previously reported to the University by AMT for the period or periods requested by the University were correct (and if such certification cannot be given

specifying the reasons why so that Parties can recalculate the relevant sums) on the following basis:

6.9.1 such firm of accountants shall be given access to and shall be permitted to examine and copy such books and records of AMT upon twenty (20) Business Days notice having been given by the University and at all reasonable times on Business Days;

6.9.2 prior to any such examination taking place, such firm of accountants shall undertake to AMT that they shall keep all information and data contained in such books and records strictly confidential and shall not disclose such information or copies of such books and records to any third person including the University, but shall only use the same for the purpose of the reviews and/or calculations which they need to perform in order to issue the certificate to the University which this Section 6.9 envisages;

6.9.3 any such access examination and certification shall occur no more frequently than once per Calendar Year and will not go back over records more than three (3) Calendar Years old unless a discrepancy is found;

6.9.4 AMT shall make available personnel to answer queries on all books and records required for the purpose of that certification;

6.9.5 the Parties agree that the results of the audit carried out by the accounting firm as formalised in a written report to be delivered to the Parties, which shall incorporate the possible re-calculation of the figures if appropriate, shall be final and binding upon the Parties; and

6.9.6 if the certification is in disagreement with the Net Sales as calculated by AMT, the Net Sales calculated by the certification shall be used for purposes of calculating any monies owed and any monies owed by one Party to the other shall be paid by that Party. The cost of the accountant shall be the responsibility of AMT if the recalculation shows that AMT's previous Net Sales figures supplied to the University to be inaccurate by more than five percent (5%) and the costs of the accountant shall be responsibility of the University otherwise.

6.10 Upon timely request by the University, AMT shall have any licensee accounting to it for royalties due the University audited under the audit provisions agreed between AMT and the licensee, and AMT shall report to the University the outcome. If there is a discrepancy identified upon such an audit the provisions of Section 6.9.6 shall apply in like manner.

6.11 All payments to the University under the terms of the Agreement are expressed to be exclusive of value added tax howsoever arising and AMT shall pay to the University in addition to those payments all value added tax for which the University is liable to account in relation to any supply made or deemed to be made for value added tax purposes to this agreement on receipt of a tax invoice or invoices from the University.

6.12 All payments made to the University under this Agreement shall be made by wire transfer to the account of the University at BANCA DI ROMA (Agenzia n. 153) Tesoreria Universitaria, Country Code IT, Swift Code BTOMITRDEST, IBAN Code IT68P0300203371000000007978, Account Number 7978, CIN Code P, ABI Code 3002, CAB Code 3371, or any other bank account that may be notified by the University to AMT from time to time.

7. CONDUCT OF THE RESEARCH PROJECT

7.1 AMT shall pay the University one hundred and twenty thousand Euro (€120,000) each year during the Research Term towards the cost of the Research Project. Thirty thousand Euro (€30,000) shall be paid in arrear on the last Business Day of each Quarter during the Research Term. Any and all payments made by AMT pursuant to this Section 7.1 shall be applied by the University solely to the Research Project.

7.2 The University shall be responsible for:

7.2.1 performing the Research Project in the Researchers Laboratory;

7.2.2 performing its tasks under the Research Project with reasonable skill and care in Good Scientific Manner and in compliance in all material aspects with all other Legal Requirements. Any animals involved in any part of the Research Project will be provided with humane care and treatment in accordance with current generally accepted veterinary practice; and

7.2.3 keeping accurate written laboratory notebooks and other records and reports of the progress of the Research Project in sufficient detail and in good scientific manner for all purposes including patent purposes. Such notebooks and other records must properly reflect all work done on the Research Project and the results achieved thereunder.

7.3 It is acknowledged that the University shall have the right to make day-to-day operational decisions as to how to discharge its obligations under the Research Project.

7.4 On or before the end of the third Quarter in each calendar year until the end of the Research Term the University shall propose a plan of the activity to be carried out by the University during the following calendar year of the Research Term. Once prepared such plan shall be submitted to the LC for discussion and comment.

7.5 Within thirty (30) days after the end of each Semester, the University shall prepare a written report covering its activities under the Research Project and pursuant to the Telethon Grant in the previous Semester and provide the same to the LC (together, the "**Semester Research Report**"). The Semester Research Report shall set out the results generated under the Research Project and pursuant to the Telethon Grant and not previously reported to the LC and any intellectual property generated, created, discovered or otherwise arising and not previously reported.

8. OWNERSHIP OF IP

8.1 As between the Parties, and subject to the licenses granted hereunder, any and all AMT IP shall be solely owned by AMT.

8.2 As between the Parties, and subject to the licenses granted hereunder, any and all University IP and Optioned IP shall be solely owned by the University.

8.3 As between the Parties, and subject to the licenses granted hereunder, any and all Research IP shall be solely owned by AMT. The University hereby assigns all its right, title and interest in the Research IP to AMT in each case from the date of conception or development.

8.4 The Parties shall do all such acts and things as are necessary to ensure title to the AMT IP, University IP, Research IP and Optioned IP is properly vested in them reflecting the ownership provisions from Sections 8.1 to 8.3, including, in the case of the University, procuring that the Researchers and other researchers in the Researchers Laboratory do all such acts and things as are necessary.

9. OPTION

9.1 AMT shall, during the Research Term and for a subsequent period of three (3) months ("**Option Period**") have an exclusive option for itself or its Affiliates to enter into an exclusive worldwide sub-licensable license under all or any part of the Optioned IP to utilize the Optioned IP for further research or to research, develop, make, have made, market and distribute, sell and have sold products covered by such Optioned IP. This option shall be exercisable upon AMT giving written notice to the University at any time during the Option Period (a "**Notice**") and on the date of such Notice the licence grant shall be deemed to have come into full force and effect on that date. The Parties shall negotiate and agree in good faith a licence agreement with details of the specific Optioned IP being licensed, financial terms including milestones and royalty payments, and any other content as might be reasonably required (each a "**Licence Agreement**").

9.2 If the Parties are unable to agree any of the terms of any License Agreement such matter shall be referred to a person suitably qualified to determine that particular matter or dispute (an "**Expert**") who shall be nominated jointly by the Parties or, failing agreement between the Parties within twenty (20) days of a written request by either Party to the other seeking to initiate the expert determination procedure, either party may request the president for the time being of the Association of the British Pharmaceutical Industry or any successor body to it to nominate the Expert.

9.3 The Parties shall with fourteen (14) days of the appointment of the Expert meet with him/her in order to agree a program for written and oral submissions.

9.4

9.4.1 In all cases the terms of appointment of the Expert by whomsoever appointed shall include:

(i) a commitment by the Parties to share equally the Expert's fee;

(ii) a requirement on the Expert to act fairly as between the Parties and according to the principles of natural justice;

(iii) a requirement on the Expert to hold professional indemnity insurance both then and for three years following the date of his/her determination;

(iv) a commitment by the Parties to supply to the Expert the submissions the subject of Section 9.3 and all such assistance, documents and information as he/she may require for the purpose of his or her determination.

(v) a commitment by the Parties that all negotiations connected with the dispute shall be conducted in confidence and without prejudice to the rights of the parties in any future proceedings.

9.4.2 The Expert shall give a written decision which shall contain a factual analysis, his/her conclusions and the reasons for his conclusions.

9.4.3 The Expert's decision shall be final and binding on the Parties (save in the case of negligence or manifest error).

9.4.4 The Parties expressly acknowledge and agree that they do not intend the reference to the Expert to constitute an arbitration within the scope of any arbitration legislation, the Expert's decision is not a quasi judicial procedure and the Parties shall have no right of appeal against the Expert's decision provided always that this shall not be construed as waiving any rights the Parties might have against the Expert for breaching his/her terms of appointment or otherwise being negligent.

9.5 If AMT does not exercise its option to any particular Optioned IP within the Option Period, the University shall be free to deal with such Optioned IP in its sole discretion.

9.6 The University shall not during the Option Period enter into any arrangement or agreement for the assignment, license, transfer or other disposal of Optioned IP, save as provided in Section hereunder, nor will the University disclose or publish the same save in accordance with Section 11.

10. IP PROSECUTION, MAINTENANCE AND INFRINGEMENT

10.1 On the Effective Date, the University shall transfer to AMT all its files relating to the Patent Application and all subsequent Patent Rights and execute any documents necessary to transfer control of the prosecution, maintenance and/or defence therefore to AMT. From then on AMT, who agrees to proceed with the European regionalisation phase of the Patent Application, shall be responsible at its own cost and expense for the prosecution, maintenance and/or defence of the Patent Application and any and all subsequent Patent Rights including conducting any necessary or desirable claims or proceedings (including but not limited to any interference, reissue or re-examination or opposition or revocation proceedings). AMT shall keep the University informed of all material developments in relation to the Patent Application and such Patent Rights and shall, upon the University's request, provide the University with copies of relevant documents related to the maintenance and defence of such Patent Rights. AMT shall consider in good faith any reasonable representation made by the University in relation to the maintenance and defence of such Patent Rights in the conduct of any proceedings in relation to such Patent Right.

10.2 In the event that AMT wishes to abandon any particular Patent Right the subject of Section 10.1 AMT shall provide the University with written notice thereof, such notice shall be given at least forty five (45) days prior to the expiration of any official substantive deadline relating to such activities. In any of such circumstances the University shall have the right to decide that the University should maintain and /or defence or continue to maintain and/or defend such Patent Rights and in such case the University shall give written notice to AMT. AMT shall upon receipt of any such notice from the University transfer to the University all its files relating to such Patent Rights and execute any documents necessary to transfer control to the University.

10.3 AMT shall be solely responsible at its own cost and expense for the preparation, filing, prosecution, maintenance, defence and enforcement of Patent Rights forming part of AMT IP and Research IP. The University shall provide all assistance reasonably necessary to facilitate the preparation, filing, prosecution, maintenance, defence and enforcement by AMT of Patent Rights forming part of the Research IP. AMT shall keep the University informed of all material developments in relation to the Research Patent Rights and shall, upon the University's request, provide the University with copies of relevant documents related to the preparation, filing, prosecution, maintenance, defence and enforcement by AMT of such Patent Rights.

10.4 The University shall be responsible at its own cost and expense for the preparation, filing, prosecution, maintenance and/or defence of the University Patent Rights (excluding the Patent Application) and Patent Rights within Optioned IP ("**UO Patent Rights**") including conducting any necessary or desirable claims or proceedings (including but not limited to any interference, reissue or re-examination or opposition or revocation proceedings). The University shall keep AMT informed of all material developments in relation to the UO Patent Rights and shall, upon AMT's request, provide AMT with copies of relevant documents related to the filing, prosecution and maintenance of the UO Patent Rights. The University shall consider in good faith any reasonable representation made by AMT in relation to the prosecution of the UO Patent Rights when making any submission to a patent office (including the scope of foreign filings) and in the conduct of any proceedings in relation to such UO Patent Rights.

10.5 In the event that the University declines to file or, having filed, declines to further prosecute, maintain and/or defend any UO Patent Rights the University shall provide AMT with written notice thereof. In the case where the University has filed but is declining to further prosecute or maintain such UO Patent Rights, such notice shall be given at least forty five (45) days prior to the expiration of any official substantive deadline relating to such activities. In any of such circumstances AMT shall have the right to decide that AMT should file, continue to file or prosecute such UO Patent Rights and in such case AMT shall give written notice to the University. The University shall upon receipt of any such notice from AMT transfer to AMT all its

files relating to the UO Patent Rights and execute any documents necessary to transfer control of such filing, prosecution and maintenance to AMT.

10.6 **Infringement**. In the case where either Party has notice or reasonable suspicion, (i) that an infringement by a third party of the University Patent Rights or the Research Patent Rights may be occurring; or (ii) it is in receipt of any declaratory judgment action alleging invalidity or non-infringement of any of the University Patent Rights or the Research Patent Rights such Party shall promptly disclose full details of the potential infringement and/or action to the other Party.

10.7 Where an infringement of the University Patent Rights is occurring and the infringement relates to the research, Development, manufacture or sale of Product, AMT shall have the first right to, but not the obligation to, at its sole cost and expense (including the payment of all attorneys' fees, legal fees, and court costs), enforce the same and prosecute any infringement in accordance with the following:

10.7.1 in such circumstances AMT shall have sole conduct of the claim and any proceedings including any counterclaim for invalidity or unenforceability or any declaratory judgment action and including the right to settle. Where AMT decides to commence proceedings in relation to the University Patent Rights it shall be entitled to require the University to join AMT as co-plaintiff. In such case the University shall provide all necessary assistance to AMT in relation to any such proceeding provided that AMT shall on demand by the University indemnify the University against the costs of such activity unless the University elects to be separately represented (which shall be at the University's discretion) in which case such separate representation shall be at the University's own cost and expense;

10.7.2 if AMT succeeds in any such infringement proceedings whether at trial or by way of settlement, any recovery by AMT in such proceedings brought by AMT shall first be used to reimburse AMT for reasonable out-of-pocket costs and legal fees incurred to conduct such proceedings. Out of any remaining compensatory damages actually received by AMT relating to infringement of the University Patent Rights, AMT shall pay to the University an amount equivalent to the royalties which would have been due

to the University on the balance as if they were Net Sales under Section 6.5, along with an accounting of the recovery and the reasonable out-of-pocket costs and legal fees; and

10.7.3 if AMT fails to take any such proceedings, the University may give AMT notice requesting AMT to take such proceedings within thirty (30) days of the date of notice and if AMT decides not to do so, the University shall be entitled to do so at its sole cost and expense (including the payment of all attorneys' fees, legal fees, and court costs) in which case it shall have sole conduct of any claim or proceedings including any counterclaim for invalidity or unenforceability or any declaratory judgment action. AMT shall provide all necessary assistance to the University in relation to such proceedings and the University shall on demand by AMT indemnify AMT against the costs of such activity, unless AMT elects to be separately represented (which shall be at AMT's discretion), in which case such separate representation shall be at AMT's cost and expense. The University shall have sole right to settle such proceedings including any counterclaim for invalidity or unenforceability. If the University succeeds in any such proceedings it shall be entitled to retain the whole of any award of costs and damages made or settlement sum paid.

10.8 Each Party shall promptly notify the other Party of any third party Patent Rights relevant to the Development or Commercialisation of Product of which it becomes aware including by making appropriate searches for these as and when it considers appropriate. The Parties' shall liaise via the LC to ensure that such searches are not being unnecessarily duplicated. To the extent possible, whilst preserving attorney-client privilege, the Parties' patent counsel shall share copies of all external and internal opinions on the likelihood of grant and/or validity of relevant third party Patent Rights and may attend the LC meeting convened to discuss third party Patent Rights.

10.9 If AMT, acting in good faith, determines that the Development or Commercialisation of Product would infringe a Valid Claim of a relevant third party Patent Right in any country and a license is required under such relevant third party Patent Rights for AMT or its Affiliate or sub-licensee to continue their activities in relation to conduct

of the Development or Commercialisation of Product, AMT shall give written notice to the University of this determination. If AMT determines that the Development or Commercialisation of Product would infringe a Valid Claim of a relevant third party Patent Right Covering Product AMT will be entitled to deduct from any sums payable to the University under Section 6.5 twenty percent (20%) of sums due to such third party for a license under such third party Patent Rights.

10.10 If either Party receives any notice, claim or proceedings from any third party alleging infringement of that third party's Patent Rights by reason of the conduct of any of the other activities hereunder the Party receiving that notice shall forthwith notify the other Party of the notice, claim or proceeding and neither Party shall make any admission of liability.

11. CONFIDENTIALITY

11.1 Except to the extent expressly authorised by this Agreement including in Section 11.2 or otherwise agreed in writing, each Receiving Party in possession of Confidential Information shall maintain such Confidential Information as confidential and use it only for the purposes of this Agreement in accordance with this Section 11. Each Party shall guard such Confidential Information using the same degree of care as it normally uses to guard its own confidential, proprietary information of like importance, but in any event no less than reasonable care. Notwithstanding the foregoing, the Receiving Party of the categories of Confidential Information identified in Section 1.9 (ii) and (v) shall be relieved of the confidentiality and limited use obligations of this Agreement to the extent that the Receiving Party establishes by written evidence that:

11.1.1 the Confidential Information was previously known to the Receiving Party from sources other than the Disclosing Party at the time of disclosure and other than under an obligation of confidentiality;

11.1.2 the Confidential Information was generally available to the public or otherwise part of the public domain at the time of its disclosure; or

11.1.3 the Confidential Information became generally available to the public or otherwise part of the public domain after its disclosure to the Receiving Party

other than through any act or omission of the Receiving Party in breach of this Agreement; or

11.1.4 the Confidential Information is acquired in good faith in the future by the Receiving Party from a Third Party who has a lawful right to disclose such information and who is not under an obligation of confidence to the Disclosing Party with respect to such information; or

11.1.5 the Confidential Information is subsequently developed by or on behalf of the Receiving Party without use of the Disclosing Party's Confidential Information.

11.2 Notwithstanding the above obligations of confidentiality and non-use a Receiving Party may:

11.2.1 disclose Confidential Information to a Competent Authority as reasonably necessary to obtain regulatory approval in a particular jurisdiction to the extent consistent with the licenses granted under terms of this Agreement; and

11.2.2 disclose Confidential Information: (i) to the extent such disclosure is reasonably necessary to comply with the order of a court; or (ii) to the extent such disclosure is required to comply with a Legal Requirement, including to the extent such disclosure is required in publicly filed financial statements or other public statements under rules governing a stock exchange (e.g., EURONEXT, the rules of the United States Securities and Exchange Commission, NASDAQ, NYSE, UKLA, or any other stock exchange on which securities issued by either Party may be listed); provided, to the extent possible bearing in mind such Legal Requirements and subject to the next subsequent sentence of this Section 11.2.2, such Party shall provide the other Party with a copy of the proposed text of such statements or disclosure five (5) Business Days in advance of the date on which the disclosure is to be made to enable the other Party to review and provide comments, unless a shorter review time is agreed. If the compliance with a Legal Requirement requires filing of this Agreement, the filing Party shall to the extent possible seek confidential treatment of portions of this Agreement from the relevant Competent Authority and shall provide the other Party with a copy of the

proposed filings at least ten (10) Business Days prior to filing for the other Party to review any such proposed filing. Each Party agrees that it will obtain its own legal advice with regard to its compliance with Legal Requirements and will not rely on any statements made by the other Party relating to such Legal Requirements; and

11.2.3 disclose Confidential Information by filing or prosecuting Patent Rights, the filing or prosecution of which is contemplated by this Agreement, without violating the above secrecy provision; it being understood that publication of such filings occurs in some jurisdictions within eighteen (18) months of filing, and that such publication shall not violate the above secrecy provision; and

11.2.4 in the case where AMT is the Receiving Party disclose Confidential Information to such Receiving Party's Affiliates, contractors (including clinical researchers) distributors, licensee's, agents, consultants, as such Receiving Party reasonably determines is necessary to receive the benefit of the licenses and rights granted or available to it under this Agreement or to fulfil its obligations pursuant to this Agreement; provided, however, any such persons must be obligated to substantially the same extent as set forth in Section 11.1 to hold in confidence and not make use of such Confidential Information for any purpose other than those permitted by this Agreement; and

11.2.5 disclose Confidential Information: (i) to its actual or potential investment bankers; (ii) to existing and potential investors in connection with an offering or placement of securities for purposes of obtaining financing for its business and to actual and prospective lenders for the purpose of obtaining financing for its business; and (iii) to a bona fide potential acquiror or merger partner for the purposes of evaluating entering into a merger or acquisition, provided, however, any such persons must be obligated to substantially the same extent as set forth in Section 11.1 to hold in confidence and not make use of such Confidential Information for any purpose other than those permitted by this Agreement; and

11.2.6 disclose Confidential Information to its legal advisers and patent lawyers for the purpose of seeking advice.

11.3 If either Party makes a publication or presentation relating to the subject matter of this Agreement, which shall only be made in accordance with the provisions of this Section 11, any scientific personnel who have been involved in the subject matter of such publication or presentation shall be named in accordance with good scientific practice. Furthermore, in the case of AMT this shall, as appropriate to the circumstances, include citation of the original publications of the inventors Dr Bozzoni and Dr De Angelis.

12. REPRESENTATIONS, WARRANTIES AND INDEMNITY

12.1 Each Party hereby represents and warrants to the other Party, as of the Effective Date, as follows:

12.1.1 Such Party (i) has the power and authority and legal right to enter into this Agreement, to perform its obligations and to grant the licenses hereunder, and (ii) has taken all necessary action on its part to authorize the execution and delivery of this Agreement and the performance of its obligations hereunder;

12.1.2 This Agreement has been duly executed and delivered on behalf of such Party and constitutes a legal and valid obligation binding upon such Party and enforceable against it in accordance with its terms;

12.1.3 The execution, delivery and performance of this Agreement by such Party do not conflict with any agreement, instrument or understanding, oral or written, to which it is a party or by which it is bound, nor violate any applicable law or regulation of any governmental body or administrative or other agency having jurisdiction over it;

12.1.4 Such Party is aware of no action, suit, inquiry or investigation instituted by any third party that questions or threatens the validity of this Agreement; and

12.1.5 All necessary consents, approvals and authorizations of all governmental authorities and other persons required to be obtained by such Party in connection with this Agreement have been obtained.

12.2 The University hereby represents and warrants to AMT as of the Effective Date that is the sole owner of the entire right, title and interest in the Patent Application and the University has not previously entered into any agreement, whether written or oral, with respect to, or otherwise assigned, licensed, transferred, conveyed or otherwise encumbered its right, title or interest in or to the Patent Application (including by granting any covenant not to sue with respect thereto).

12.3 Except with respect to third party claims the subject of Section 12.4, neither Party shall be liable to the other in contract, tort, negligence, breach of statutory duty or otherwise for any loss, damage, costs or expenses of any nature whatsoever incurred or suffered by the other or its Affiliates:

12.3.1 of a direct nature where the same is a loss of turnover, profits, business or goodwill; or

12.3.2 an indirect or consequential or punitive nature, including any indirect or consequential economic loss or other indirect or consequential loss of turnover, profits, loss of enterprise value, business or goodwill or otherwise.

12.4 AMT shall defend, indemnify and hold the University harmless from and against any losses arising out of third party claims, suits or demands based on alleged or actual bodily injury or death resulting from the Development or Commercialisation of Product by AMT, its Affiliates or sublicensees. Losses shall not include any liability, claims, lawsuits, losses, damages, costs or expenses to the extent the same are determined to be the result of any actions or omissions of the University or their Affiliates, directors, officers, employees and agents.

12.5 An indemnified person under Section 12.4 ("**Indemnified Party**") shall give the indemnifying party under Section 12.4 or ("**Indemnifying Party**") prompt written notice of any Loss or discovery of any relevant Third Party claim ("**Third Party Claim**") upon which such Indemnified Party intends to base a request for indemnification under Section 12.4 (an "**Indemnification Claim Notice**"). Where required the Indemnifying Party shall promptly send a copy of the Indemnification Claim Notice to its relevant insurers and shall permit them to exercise their rights of subrogation and hereafter in this Section 12.5 "Indemnifying Party" shall be deemed to include any such insurers. In no event shall the Indemnifying Party be liable for

any Loss that results from any delay in providing the Indemnification Claim Notice. Each Indemnification Claim Notice shall contain a description of the claim and the nature and amount of the Loss claimed (to the extent that the nature and amount of such Loss is known at such time). The Indemnified Party shall furnish promptly to the Indemnifying Party copies of all correspondence, communications and official documents (including court documents) received in respect of any such loss. For the avoidance of doubt, all indemnification claims in respect of a Party, its Affiliates or their respective directors, officers, employees and agents (each, an "**Indemnitee**") shall be made solely by a Party to this Agreement or its insurers.

12.6 The obligations of an Indemnifying Party under this Section 12 shall be governed by and contingent upon the following:

12.6.1 At its option, the Indemnifying Party may assume control of the defense of any Third Party Claim (which, for the avoidance of doubt, shall include the conduct of all dealings with such Third Party) by giving written notice to the Indemnified Party within fourteen (14) days after the Indemnifying Party's receipt of an Indemnification Claim Notice. The assumption of control of the defense of a Third Party Claim by the Indemnifying Party shall not be construed as an acknowledgement that the Indemnifying Party is liable to indemnify any Indemnitee in respect of the Third Party Claim, nor shall it constitute a waiver by the Indemnifying Party of any defenses it may assert against any Indemnified Party's claim for indemnification.

12.6.2 Upon the assumption of the control of the defense of a Third Party Claim by the Indemnifying Party:

(a) subject to the provisions of Section 12.6.3, it shall have the right to and shall assume sole control and responsibility for dealing with the Third Party and the Third Party Claim, including the right to settle the claim on any terms the Indemnifying Party chooses, but at all times in accordance with the provisions of Section 12.6.4;

(b) if it chooses, the Indemnifying Party may appoint as counsel in the defense of the Third Party Claim any law firm or counsel selected by the Indemnifying Party; and

(c) except as expressly provided in Section 12.6.3, the Indemnifying Party shall not be liable to the Indemnified Party for any legal expenses subsequently incurred by such Indemnified Party or any Indemnitee in connection with the analysis, defense or settlement of the Third Party Claim. In the event that it is ultimately determined that the Indemnifying Party is not obligated to indemnify, defend or hold harmless an Indemnitee from and against the Third Party Claim, the Indemnified Party shall reimburse the Indemnifying Party for any and all costs and expenses, including lawyers' fees and costs of suit, and any Losses incurred by the Indemnifying Party in its defense of the Third Party Claim with respect to such Indemnified Party or Indemnitee.

12.6.3 Without limiting the remainder of this Section 12.6, any Indemnitee shall be entitled to participate in, but not control, the defense of a Third Party Claim by having its views regularly solicited by the Indemnifying Party and, where proceedings are commenced, to retain counsel of its choice for such purpose; provided, however, that such retention shall be at the Indemnitee's own expense unless, (a) the Indemnifying Party has failed to assume the defense and retain counsel in accordance with Section 12.6.1 (in which case the Indemnified Party shall control the defense), or (b) the interests of the Indemnitee and the Indemnifying Party with respect to such Third Party Claim are sufficiently adverse to prohibit the representation by the same counsel of both Parties under any Legal Requirement, ethical rules or equitable principles.

12.6.4 With respect to any losses relating solely to the payment of money to the Third Party to settle the Third Party Claim and that will not result in the Indemnified Party or the Indemnitee becoming subject to injunctive relief, and as to which the Indemnifying Party shall have acknowledged in writing the obligation to indemnify the Indemnitee under Section 12.6.1, the Indemnifying Party shall have the sole right to enter into any such settlement including any consent judgment, on such terms as the Indemnifying Party, in its sole discretion, shall deem appropriate. With respect to all other Losses or where the Indemnified

Party will be subject to injunctive relief, where the Indemnifying Party has assumed the defense of a Third Party Claim in accordance with Section 12.6.1, the Indemnifying Party shall have authority to consent to the entry of any judgment, enter into any settlement or otherwise dispose of such Losses, provided that it obtains the prior written consent of the Indemnified Party (which consent shall not be unreasonably withheld or delayed).

12.6.5 If the Indemnifying Party chooses not to take control of the defense or prosecute any Third Party Claim, the Indemnified Party shall retain control of the defense thereof but no Indemnified Party or Indemnitee shall admit any liability with respect to, or settle, compromise or discharge, any such Third Party Claim without the prior written consent of the Indemnifying Party, which consent shall not be unreasonably withheld or delayed. The Indemnifying Party shall not be liable for any settlement or other disposition of Losses by an Indemnified Party or an Indemnitee under such a Third Party Claim that is reached without the written consent of the Indemnifying Party.

12.6.6 If the Indemnifying Party chooses to control the defense of any Third Party Claim, the Indemnified Party shall, and shall cause each other Indemnitee to, reasonably cooperate in the defense thereof and shall furnish such records, information and testimony, provide such witnesses and attend such conferences, discovery proceedings, hearings, trials and appeals as may be reasonably requested in connection therewith. Such cooperation shall include access during normal business hours by the Indemnifying Party to, and reasonable retention by the Indemnified Party of, records and information that are reasonably relevant to such Third Party Claim, and making the Indemnified Party, the Indemnitees and its and their employees and agents available on a mutually convenient basis to provide additional information and explanation of any records or information provided, and the Indemnifying Party shall reimburse the Indemnified Party for all of its related reasonable out-of-pocket expenses.

12.6.7 Except as expressly provided above, the reasonable and verifiable costs and expenses, including fees and disbursements of counsel, incurred by the Indemnified Party where it participates in the defense under Section 12.6.3 or

Section 12.6.5 shall be reimbursed on a Quarterly basis by the Indemnifying Party, without prejudice to the Indemnifying Party's right to contest the Indemnified Party's right to indemnification and subject to refund in the event the Indemnifying Party is ultimately held not to be obligated to indemnify the Indemnified Party.

13. TERM AND TERMINATION

13.1 Subject to the other provisions of this Section 13 this Agreement shall come into force on the Effective Date and expire on a country by country basis when no further payment is due from AMT to the University hereunder in relation to sales of Product in that country (the "**Term**") in which case, AMT's license under Section 2 becomes perpetual, irrevocable and fully paid-up on a country-by-country basis. Any such expiry shall not affect any Licence Agreement entered into pursuant to this Agreement prior to the date of termination.

13.2 AMT shall have the right at any time to give sixty (60) days' notice of termination of this Agreement in writing to the University if in AMT's sole opinion AMT decides not to continue with the research, Development or Commercialisation of Product. This Agreement shall terminate upon expiration of such sixty (60) day notice period. In this case AMT shall provide the University with a final progress report in relation to Development of the Product.

13.3 Each of the Parties (the "**Termination Party**") shall have the right to terminate this Agreement with immediate effect upon giving written notice of termination to the other Party (the "**Defaulting Party**") upon any material breach (which in the case of AMT shall include breach of Sections 6.1 to 6.6) of this Agreement by the other Party that has not been cured by the Defaulting Party within sixty (60) days after receipt of notice of such material breach, unless (i) on or before such date the Defaulting Party is diligently undertaking substantive and progressive efforts to remedy such breach and such breach is in fact remedied as soon as reasonably possible, or (ii) the Defaulting Party dispute that a material breach has occurred and submits them to dispute resolution under Section 14.5 (in which case there shall be no termination of this Agreement pending the outcome of such dispute resolution).

13.4 The University shall have the right to terminate this Agreement on written notice to AMT in the event of the occurrence of an Insolvency Event in relation to AMT .

13.5 If either Party terminates this Agreement under Section 13.3 all licenses and other rights granted hereunder to the Defaulting Party shall cease and all the terms of this Agreement shall terminate save for Sections 8, 11, 12, 13.5, and 14 which shall survive termination. If the University is the Defaulting Party AMT's license under Section 2 becomes perpetual, irrevocable and fully paid-up on a country-by-country basis and Sections 9 and 10 shall also survive termination. In such a case the University shall promptly provide AMT with a final Semester research report and shall promptly deliver up to AMT all Research IP in the University's possession and/or control. If the University terminates this Agreement under Sections 13.3 or 13.4 all licenses and other rights granted hereunder to AMT shall cease and all the terms of this Agreement shall terminate save for Sections 8, 11, 12, 13.5, and 14 which shall survive termination. Upon the termination of expiry of this Agreement for any reason all existing Licence Agreements shall remain in full force and effect.

13.6 Termination of this Agreement for whatever reason shall not terminate AMT's obligation to pay all milestones and royalties with respect to Products which shall have accrued hereunder, prior to termination.

14. MISCELLANEOUS

14.1 Each of the Parties shall do all such acts and things and execute all such deeds and documents as may be necessary or desirable for them to perfect the title of the other Party in any Patent Rights, Know How or Materials as necessary to implement the provisions of this Agreement.

14.2 Neither Party shall without the prior written consent of the other, such consent not to be unreasonably withheld, assign the benefit and/or burden of this Agreement save that AMT may, without the consent of the University, assign this Agreement and its rights and obligations hereunder to an Affiliate or in connection with the transfer or sale of all or substantially all of its business related to this Agreement whether in a merger, consolidation, acquisition or similar transaction. Any permitted assignee shall assume all obligations of AMT under this Agreement and any purported assignment or transfer in violation of this Section 14.2 shall be void.

14.3 Any amendment or modification of this Agreement must be in writing and signed by both parties.

14.4 If a Party is unable to perform any of its obligations under this Agreement due to an event of force majeure as determined by the common law such Party shall be excused such performance (but only such performance) during the period of such force majeure event.

14.5 The validity, construction and interpretation of this Agreement and any determination of the performance which it requires shall be governed by the laws of England and Wales. All disputes between the Parties arising out of the circumstances and relationships contemplated by this Agreement including disputes relating to the validity, construction or interpretation of this Agreement, and including disputes relating to pre-contractual representations, shall first be referred to the respective Chief Executive Officer of AMT and the Technological Transfer Office of the University for resolution, but if the Chief Executive Officer of AMT and the Technological Transfer Office of the University are unable to resolve the dispute within thirty (30) days of its referral, the dispute shall be subject to the jurisdiction of the English Courts.

14.6 Save as expressly provided in this Agreement nothing herein takes away from either Party or constitutes a waiver by either Party of any of its rights or remedies under common law, statute or otherwise.

14.7 This Agreement constitutes the entire agreement and understanding between the Parties and supersedes all prior oral or written understandings, arrangements, representations or agreements between them relating to the subject matter of this Agreement provided that this does not remove any right of action by either Party in respect of any fraudulent misrepresentation, fraudulent concealment or other fraudulent action.

14.8 Should any provision of this Agreement be held invalid, illegal or unenforceable, by a court or other entity of competent jurisdiction, such provision shall be considered void. All other provisions, rights and obligations shall remain enforceable.

14.9 All formal notices to be given pursuant to this agreement shall be in writing and shall be delivered by hand to the address of the Parties set out above (or such other address as may be notified by a Party to the other from time to time) with a confirmation copy being sent by post. Notices shall be deemed to have been received at the time of delivery by hand.

14.10 The activities of the Parties contemplated pursuant to this Agreement shall not constitute a partnership and neither Party has the authority to bind the other Party in anyway except provided in this Agreement.

14.11 Each Party shall bear its own legal costs and other expenses incurred in the negotiation, preparation, execution and implementation of this Agreement.

14.12 Any press releases to be made by either Party relating to this Agreement will require the approval of the other Party.

14.13 Only the Parties and their successors and permitted assignees shall have a right to enforce any provision of this Agreement and no other person shall have any rights to enforce a term of this Agreement which confers a benefit on that person.

IN WITNESS WHEREOF, and intending to be legally bound, the Parties have caused this Agreement to be executed by their duly authorized representatives as of the Effective Date.

SIGNED by

AMSTERDAM MOLECULAR THERAPEUTICS B.V.

.....A. J. Gringeri.....

Name: ...Anthony J. Gringeri, Ph.D....

Title: ...Chief Operating Officer.....

Date: ...22 April 2008.....

SIGNED by

LA SAPIENZA UNIVERSITY

The Rector

.....R. T. Guarini.....

Name: Prof. Renato Guarini

Title: The Rector

Date: .....09 MAG. 2008.....

UNIVERSITÀ DEGLI STUDI DI ROMA "LA SAPIENZA"

VERBALE DELLA COMMISSIONE TECNICA BREVETTI
7 MARZO 2008

Il giorno 7 marzo 2008 alle ore 12.00, nella stanza del Senato del palazzo del Rettorato, è convocata la Commissione Tecnica Brevetti per discutere il seguente ordine del giorno:

1. Comunicazioni
2. Esame e valutazione delle seguenti domande di brevetto:
   2.1 Nuova proposta di invenzione brevettabile- prof. Bruno Scrosati
   2.2 Domanda di Brevetto RM2007A000228- prof. Bruno Scrosati- estensione
   2.3 Domanda di Brevetto RM2007A000209- prof. Bianco- estensione
   2.4 Domanda di Brevetto RM2007A000182- prof.ssa Negri- estensione
   2.5 Nuova proposta di invenzione brevettabile- dott. Leonardo Mattiello
3. Esame eventuali proposte di cessione di invenzione brevettabile pervenute dopo la presente convocazione.
   3.1 Nuova proposta di invenzione brevettabile- prof. Federico Bordi
4 Varie ed eventuali
   4.1 Proposta di fornitura di prototipi di dispositivo medico della MED marine srl- prof. De Vincentiis
   4.2 Proposta di Cessione di invenzione brevettabile- proff. Trifiletti, D'Andrea

Presenti: Misiti, Libertini, Gulino, Carlucci Aiello, Scarano e Pennacchini
Assume le funzioni di segretario la dott.ssa Pennacchini

1. Comunicazioni

- Patent applications RM2002A000253 del 8.5.2002 e PCT IT/03/000273 (EP: WO 03/095647) del 6.5.2003 di proprietà dell'Universita': contratto di licenza e sperimentazione.

Su iniziativa del Presidente prof. Domenico Misiti, è stata invitata a conferire la prof.ssa Bozzoni in merito al contratto in oggetto, sul quale a tutt'oggi sono ancora in corso le trattative con la AMT, con la quale in data 21.12.07 è stato sottoscritto un Term sheet a fronte del riconoscimento un diritto di opzione.
Vengono preliminarmente sottolineati dal Presidente, e condivisi da tutti componenti della Commissione, gli aspetti di alto contenuto morale ed etico sottesi a tale operazione negoziale e relativi alla possibilità di giungere alla messa a punto di una terapia genica efficace contro la malattia di Duchenne; per opinione condivisa si sottolinea l'opportunità che, al di là degli aspetti amministrativi, giuridici e contrattuali, di tali valori si faccia, almeno in parte, carico l'Università, per quanto almeno attiene la propria *mission* istituzionale.
La discussione si incentra sulla ripartizione del corrispettivo iniziale di € 300.000.00 destinato a finanziare sia l'acquisizione in licenza esclusiva dei diritti brevettuali, sia il trasferimento del know how da mettere a punto (e trasferire alla AMT) da parte del gruppo di ricerca della Prof.ssa Bozzoni.
La prof.ssa Bozzoni sottolinea la necessità di poter acquisire ed avere a disposizione le necessarie risorse finanziarie per ottemperare all'obbligo sopra citato e completare i necessari steps di ricerca.
La messa a punto della molecola da fornire alla AMT a fronte del corrispettivo previsto implica un impegno finanziario quantificabile in € 90.000,00.

Si prende in considerazione l'opportunità di reinvestire (una volta garantita *in primis* la copertura dei costi brevettuali passati e futuri) una quota parte di utili netti dell'Università per il completamento e la finalizzazione della sperimentazione, così come previsto dall'art.7 del vecchio Regolamento Brevetti del 13.12.1999 (sotto la cui disciplina rientra la domanda di brevetto di cui in oggetto), ai sensi del quale gli utili netti derivanti da contratti di licenza e simili che confluiscono nel Fondo Brevetti, sono destinati al finanziamento di attività di ricerca e brevettazione.
Si apre una discussione in tal senso, attraverso la quale emerge una posizione condivisa incentratasi sull'ipotesi di garantire la disponibilità della somma necessaria innanzitutto attraverso la messa a disposizione da parte del Dipartimento di Genetica e Biologia Molecolare della quota di utili di propria spettanza (pari al 20% degli utili netti totali) quale forma di compartecipazione alle spese della ricerca in corso; in secondo luogo garantire, attraverso il reinvestimento di una quota degli utili netti spettanti all'Università pari alla quota mancante, il raggiungimento della somma € 90..000,00 al fine di consentire di addivenire con le risorse necessarie e nei tempi previsti alla produzione della molecola di cui al progetto di sperimentazione.
La Commissione al termine della discussione, esprime parere favorevole sulla bozza di contratto di licenza e sperimentazione con la Società AMT e sugli aspetti economico-finanziari dello stesso, e ferma restando l'esigenza, sancita dal Regolamento, di garantire il ripianamento dei costi diretti e indiretti sostenuti e da sostenere (fino al rilascio del brevetto nei paesi indicati nel contratto) per gli oneri di brevettazione, approva all'unanimità la proposta di garantire attraverso la messa a disposizione della quota di utili del Dipartimento e il reinvestimento di una quota di utili netti spettanti all'Università, la disponibilità complessiva di € 90.000,00 per la produzione della molecola da fornire alla Società AMT attraverso il completamento del protocollo di sperimentazione. La Commissione dà altresì mandato all'Ufficio di quantificare i costi sostenuti, e da sostenere attraverso la richiesta di apposito preventivo al consulente brevettale, al fine di poter quantificare conseguentemente i movimenti finanziari descritti sopra.
Letto ed approvato seduta stante.

……omissis……

Non essendoci null'altro da discutere, la riunione si chiude alle ore 14.30. La prossima è convocata per il giorno 9 aprile 2008 alle ore 11.00.

F.to: Il Presidente
(prof. Domenico Misiti)

F.to: Il Segretario
(dott.ssa Francesca Pennacchini)

L'AMMINISTRAZIONE CENTRALE DELL' UNIVERSITA' DEGLI STUDI "LA SAPIENZA" DI ROMA
E
IL DIPARTIMENTO DI GENETICA E BIOLOGIA MOLECOLARE "C. DARWIN"

PREMESSO

- che l'Università degli Studi di Roma "La Sapienza" è titolare, in via originaria, del **BREVETTO ITALIANO IT1333549 DEL 04/05/2006** e relativa estensione all'estero n. PCT IT/03/000273 (ep: WO 03/095647) del 06/05/2003, inventori: prof.ssa Irene Bozzoni, dott.ssa Fernanda Gabriella De Angelis;

- che in data 21/12/2007, l'Università ha sottoscritto con la Società Amsterdam Molecular Therapeutics B.V. (A.M.T.) un Term Sheet concordato tra l'Università stessa e la predetta Società, con il quale sono stati predeterminati i contenuti fondamentali per la conclusione di un successivo contratto misto di licenza, sviluppo e sperimentazione;

- che, in conformità ai termini ed alle condizioni di cui al succitato Term Sheet, è stato stipulato tra l'Università degli Studi di Roma "La Sapienza" (d'ora in avanti "La Sapienza") e la Società Amsterdam Molecular Therapeutics B.V. (d'ora in avanti "A.M.T.") il definitivo contratto di licenza allegato al presente atto quale parte integrante e sostanziale dello stesso (d'ora in avanti "Contratto" - all. n. 1) per la licenza esclusiva, con facoltà di sublicenza, dei diritti di sfruttamento sui brevetti di cui sopra e per il finanziamento della fase di sviluppo e sperimentazione;

- che il "Contratto" costituisce un *unicum* negoziale, di natura mista, in quanto disciplinante sia la concessione in licenza dei diritti di sfruttamento industriale della proprietà intellettuale, sia il finanziamento della fase di sviluppo e sperimentazione;

- che il "Contratto" prevede un corrispettivo iniziale di € 300.000,00 (trecentomila) a fronte della concessione dei diritti di sfruttamento della proprietà intellettuale appartenenti all'Università e del trasferimento del know-how da sviluppare tramite il protocollo di sperimentazione da parte del gruppo di ricerca coordinato dalla prof.ssa Irene Bozzoni, afferente al Dipartimento di Genetica e Biologia Molecolare "C. Darwin" dell'Università "La Sapienza";

- che il "Contratto" prevede, altresì, una serie di finanziamenti successivi di ammontare complessivo pari ad € 4.700.000,00, la cui corresponsione da parte di A.M.T. è condizionata all'avanzamento del protocollo di sperimentazione ed ai risultati della stessa così come indicati nel "Contratto" medesimo nonché il pagamento di ulteriori € 360.000,00 (suddivisi in tre rate annuali da € 120.000,00 ciascuna) destinati al finanziamento della ricerca di base su nuove mutazioni genetiche riguardanti la malattia di Duchenne condotta presso il Dipartimento predetto dal gruppo di ricerca della prof.ssa Bozzoni;

- che, in particolare, le domande di brevetto di cui sopra ricadono nell'ambito di applicazione del Regolamento Brevetti dell'Università degli Studi di Roma "La Sapienza" approvato il 13/12/99 (attuativo del R.D. 29/06/1939, n. 127, L.B.I.), il quale stabilisce all'art. 7 un apposito sistema di ripartizione dei corrispettivi (utili) derivanti dalla cessione in licenza dei brevetti dell'Università - al netto degli oneri di brevettazione posti a totale carico del Bilancio dell'Università - nella seguente misura: 30% agli autori dell'invenzione; 20% alla Struttura di appartenenza degli autori medesimi; 50% all'Amministrazione Centrale dell'Università destinato al finanziamento di attività di ricerca e brevettazione;

- che lo stesso art. 7 prevede, infatti, che i costi diretti e indiretti sostenuti e da sostenersi per i suddetti oneri di brevettazione debbano, in primo luogo, trovare la loro copertura economica nei corrispettivi totali (utili lordi) rivenienti dalla cessione in licenza dei brevetti universitari;

- che pertanto, nel caso specifico, il corrispettivo inizialmente versato da A.M.T. all'atto della sottoscrizione del "Contratto" stesso, pari ad € 300.000,00, deve essere destinato *in*

primis a ripianare i costi di brevettazione già sostenuti dall'Amministrazione universitaria per un importo pari a € 19.596,30;

- che, quindi, il corrispettivo residuo (utile netto) di cui al "Contratto" di che trattasi è pari ad € 280.403,70 totali (€ 300.000,00 - € 19.596,30) e dovrà essere ripartito nella misura percentuale sopra indicata, secondo i seguenti importi: € 84.121,11 agli autori dell'invenzione; € 56.080,74 al Dipartimento di Genetica e Biologia Molecolare "C. Darwin", quale struttura di afferenza della Prof.ssa Bozzoni e della Dott.ssa Fernanda Gabriella De Angelis; restanti € 140.201,85 all'Amministrazione Centrale dell'Università;

- che, cionondimeno, si prospetta la necessità di disporre di ulteriori risorse finanziare in misura pari ad € 90.000,00 (novantamila), così quantificate dalla prof.ssa Bozzoni come indispensabili per la messa a punto della molecola da sottoporre al successivo protocollo di sperimentazione e per il trasferimento del relativo know-how (adempimento a cui è subordinata l'entrata in licenza e l'ottemperanza agli obblighi contrattuali con essa assunti);

- che, in ragione di quanto sopra esposto, si rende necessaria, nell'ottica della compartecipazione, la messa a disposizione da parte del Dipartimento di Genetica e Biologia Molecolare "C. Darwin" dell'intera quota di utili di propria spettanza – pari ai succitati € 56.080,74 – e da parte dell'Amministrazione Centrale dell'Università di una quota di utili di propria spettanza - pari ad € 33.919,26 - da trasferirsi al su nominato Dipartimento per il conseguimento dello scopo contrattuale;

Tutto ciò premesso e considerato

con il presente accordo e nel rispetto delle premesse, costituenti parte integrante dell'accordo stesso,

Il Dipartimento di Genetica e Biologia Molecolare "C. Darwin" si impegna a:

1) reinvestire e vincolare, a favore del progetto di ricerca per i fini di cui in premessa, l'intera quota di utili di propria spettanza derivanti dal contratto di licenza e di sperimentazione del **BREVETTO ITALIANO IT1333549 DEL 04/05/2006** e relativa estensione all'estero n. PCT IT/03/000273 (ep: WO 03/095647) del 06/05/2003, sottoscritto dall'Università con la Società A.M.T. in data 09/05/2008 ed ammontanti a € 56.080,74;

2) garantire al gruppo di ricerca coordinato dalla prof.ssa Irene Bozzoni la disponibilità complessiva di € 90.000,00 a seguito dell'avvenuto introito della somma di € 33.919,26 trasferita dalla Amministrazione Centrale dell'Università al momento dell'introito, da parte di quest'ultima, dell'intero up-front contrattuale per i motivi tutti in premessa indicati;

3) gestire il "Contratto" e i finanziamenti ricevuti per lo sviluppo delle attività di ricerca e sperimentazione nello stesso descritte, nel rispetto dei termini e delle condizioni previste dallo stesso, assumendosi pertanto la responsabilità contrattuale del medesimo e vincolando tali finanziamenti al raggiungimento degli obiettivi di sperimentazione in esso descritti.

L'Amministrazione Centrale dell'Università si impegna a:

1) reinvestire una quota parte di utili di propria spettanza, derivanti dall'operazione negoziale con A.M.T., per una somma pari a € 33.919,26 per le motivazioni di cui in premessa ed a trasferirla al Dipartimento di Genetica e Biologia Molecolare "C. Darwin";

2) trasferire al Dipartimento sopra citato i successivi finanziamenti corrisposti da A.M.T. (per un totale previsto pari a € 4.700.00,00), dietro presentazione in copia degli stati di avanzamento presentati dal gruppo di ricerca coordinato dalla Prof.ssa Bozzoni alla predetta Società nel rispetto degli impegni assunti con la stessa A.M.T.;

3) trasferire al medesimo Dipartimento il finanziamento di € 360.000,00, da corrispondersi da parte di A.M.T. all'Università in tre rate annuali, per le attività di ricerca finalizzate allo studio di nuove mutazioni genetiche riguardanti la malattia di Duchenne.

I termini e le condizioni del presente atto, unitamente alle premesse tutte sopra esposte, sono da ritenersi requisiti essenziali per la validità ed efficacia del contratto sottoscritto tra l'Università e A.M.T. il 09/05/2008 e, per l'effetto, devono intendersi quali parti integranti e sostanziali del contratto stesso.

Elenco allegati:

1) Contratto di licenza e sperimentazione di A.M.T. del 09/05/08;
2) Delibera del C.d.A. dell'Università degli Studi di Roma "La Sapienza" dell'11/12/2007.

Il presente atto viene sottoscritto in Roma il e redatto in duplice copia originale.

Il Dipartimento di Genetica e Biologia	**L'Amministrazione Centrale dell'Università**
Molecolare "C. Darwin"	**degli Studi "La Sapienza" di Roma**
IL DIRETTORE	**IL RETTORE**

Irene Bozzoni
Dipartimento di Genetica e Biologia Molecolare

Attività di ricerca relativa al licence and research agreement con la "Amsterdam Molecular Therapeutics" (AMT)

1) preparazione dell' "University material"

2) attività relativa al "research agreement" con AMT

Premessa

La Distrofia Muscolare di Duchenne (DMD) è una malattia genetica dovuta a una mutazione nel gene codificante per la distrofina. Essa è una patologia degenerativa del tessuto muscolare e colpisce principalmente i maschi. Scoperta nel 1868 dal medico francese Duchenne da cui prende il nome, la malattia comporta una progressiva degenerazione dei muscoli costringendo, in breve tempo, all'uso della sedia a rotelle e successivamente alla morte per compromissioni cardio-circolatorie.
Trattandosi di una malattia causata da un solo gene, si pensava inizialmente di poterla trattare mediante un approccio classico di terapia genica, ovvero mediante la somministrazione di un gene "sano" alle cellule muscolari. Purtroppo a causa delle dimensioni del gene della distrofina, che è il più grande tra i nostri geni, tale approccio si è rivelato fin dall'inizio impossibile da perseguire.
Nella ricerca di un approccio alternativo, il mio gruppo di ricerca è riuscito a mettere a punto una nuova strategia che ripara il prodotto del gene malato, anzichè tentare di sostituirlo con una copia sana. Questa strategia si basa sul principio di poter correggere il difetto genico non operando a livello del DNA bensì modificando l'RNA: attraverso l'uso di molecole 'antisenso' che riconoscono la regione contenente la mutazione nel precursore dell'mRNA, si riesce ad impedirne l'inclusione nell'RNA messaggero interferendo con la reazione di splicing. Tale tecnica è stata definita **EXON SKIPPING**. Il risultato finale è la produzione di un RNA codificante per una proteina più corta di quella prodotta nei muscoli delle persone sane, ma ancora funzionante.
Tale procedura è stata oggetto del brevetto di cui si sta trattando la cessione alla ditta AMT.
Attraverso la strategia dell'exon skipping siamo riusciti a curare topolini malati di Distrofia Muscolare di Duchenne. Abbiamo dimostrato che iniettando nei topi un virus AAV, in cui sono clonati i geni che producono le molecole antisenso, questo si distribuisce in tutti i muscoli dove, viene recuperata la sintesi della proteina distrofina. Ciò è vero anche nel cuore e nel diaframma che sono i distretti muscolari più gravemente compromessi dalla malattia. I topolini trattati in laboratorio hanno beneficiato della terapia fino a tempi molto lunghi dopo un singolo trattamento: l'analisi compiuta nell'arco di 18 mesi dall'iniezione ha permesso di dimostrare che i muscoli trattati migliorano sia in termini di forza della contrazione, sia in termini di integrità. Inoltre si è visto che il trattamento conferisce un notevole beneficio all'animale, che manifesta un importante miglioramento delle prestazioni muscolari (Denti et al., 2006 **Proc. Natl. Acad. Sci. USA,** 103: 3758-3763.

Interesse di Amsterdam Molecular Therapeutics

I risultati promettenti ottenuti nel modello animale aprono interessanti prospettive terapeutiche nell'uomo. La AMT è l'unica Biotec al mondo a possedere la tecnologia relativa all'uso di vettori AAV nella terapia genica applicata all'uomo. Tale ditta ha diversi composti che sono già in fase di sperimentazione clinica. Pertanto la AMT vuole entrare nel campo della Terapia Genica della DMD e ha valutato che il metodo dell'exon skipping è quello preferibile tra i diversi approcci di terapia genica disponibili.

1) preparazione dell' "University material"

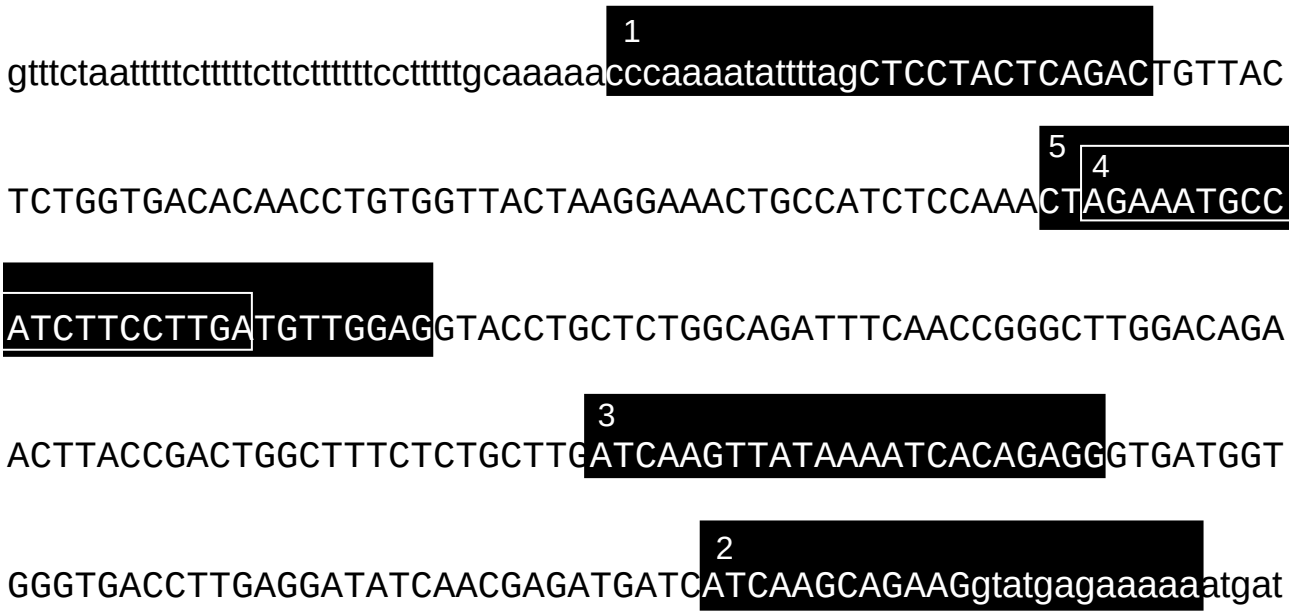
L'accordo con AMT, in questa prima fase, è quello di acquisire oltre alla licenza del brevetto anche la prima molecola da avviare verso la sperimentazione pre-clinica e clinica. Le mutazioni DMD d'elezione sono quelle che possono essere curate con lo skipping dell'esone 51. Questo permetterebbe di curare circa il 15% di tutte le mutazioni DMD note.
L'"**University material**" a cui si fa' riferimento nella proposta di license and research agreement con la ditta AMT consiste in un costrutto genico codificante per un RNA antisenso in grado di indurre efficiente skipping dell'esone 51 del trascritto primario per la distrofina umana.
Poiché questa molecola sarà quella prescelta per la registrazione all'EMEA e per le successive sperimentazioni pre-cliniche, questa fase è particolarmente critica in quanto si deve avere la certezza di aver selezionato il costrutto più attivo tra una collezione di candidati. Oltre alla ideazione e produzioni di questi costrutti si deve procedere alla loro analisi funzionale in cellule muscolari di pazienti affetti dalla patologia e portatori di diverse mutazioni, tutte però curabili con lo skipping dell'esone 51.
Il mio laboratorio aveva precedentemente ottenuto un costrutto che induceva skipping dell'esone 51 (De Angelis et al., 2002 **Proc. Natl. Acad. Sci. USA**, 99:9456-61), questo però consisteva in sequenze antisenso solo contro la giunzione di splicing 5' e l'attività risultante non era elevata.
Nel corso della sperimentazione nel modello animale del topo distrofico, abbiamo scoperto che solo costruendo antisenso contro ambedue le giunzioni di splicing si riusciva ad ottenere un efficiente exon skipping.
Pertanto lo scopo dell'attività che stiamo svolgendo dallo scorso mese di giugno, ovvero da quando la ditta AMT ha mostrato interesse al nostro prodotto, è stata quello di ideare, costruire e analizzare l'attività di nuovi costrutti antisenso specifici contro più di una sequenza di splicing.

Piano sperimentale

Ideazione e produzione dei costrutti antisenso

La figura 1 mostra la sequenza dell'esone 51 del gene della Distrofina umana. In questo sono evidenziate le giunzioni di splicing al 5' e 3' e altre sequenze importanti per lo splicing, quali la sequenza del branch point e vari enhancer di splicing (ESE). Tali sequenze, note come polypurine-rich exon recognition sequence (ERSs) o exonic splicing enhancer (ESEs), rappresentano i siti di legame di una classe di proteine ricche in serina/ arginina (proteine SR); il legame di queste proteine, nei tessuti in cui sono espresse, promuovono il riconoscimento di quell'esone. L'esclusione di un esone contenente un ESE puo quindi essere indotta costruendo opportuni RNA antisenso che legandosi a tali sequenze inibiscano il legame delle proteine SR. E' nostro intento verificare se anche questo tipo di approccio possa essere impiegato nel caso della DMD in congiunzione con l'uso di antisenso contro le giunzioni di splicing. Pertanto in base a quanto da noi dimostrato e quando indicato da altri, abbiamo pensato di confrontare l'attività di exon skipping di 6 diversi antisenso indicati in calce alla figura 1.

FIGURA 1



Nome del costrutto antisenso	Sequenza bersaglio	Sequenza della porzione antisenso nei costrutti
U1#51 5'3'	1+2	TTTTTCTCATACCTTCTGCTTGATGTCTGAGTGGAGCTAAAATATTTTGGG
U1#51 5'ESE	2+3	TTTTTCTCATACCTTCTGCTTGATCCTCTGTGATTTTATAACTTGAT
U1#51 AON1	4	TCAAGGAAGATGGCATTTCT
U1#51 AON1+2	3+4	CCTCTGTGATTTTATAACTTGATTCAAGGAAGATGGCATTTCT
U1#51 3'AON 1	1+4	TCAAGGAAGATGGCATTTCTGTCTGAGTAGGAGCTAAAATATTTTGGG
U1#51 B30	5	CTCCAACATCAAGGAAGATGGCATTTCTAG

Sono indicate in maiuscolo la sequenza dell'esone 51 e in minuscolo quella delle porzioni introniche fiancheggianti; le sequenze target degli antisenso sono riquadrate in nero e numerate da 1 a 5.

Reclutamento pazienti e ottenimento del materiale biologico

Una parte importante di questa parte di progetto riguarda il reclutamento di pazienti DMD con mutazioni diverse tra loro ma ugualmente curabili con lo skipping dell'esone 51. Grazie alla collaborazione con l'associazione dei malati (**Parent Project Onlus**) abbiamo avuto accesso al loro registro dei pazienti e di alcuni di questi sono disponibili biopsie muscolari. Tra quelli di cui non sono disponibili biopsie abbiamo a tutt'oggi reclutato 5 bambini che sono stati sottoposti a prelievo sottocutaneo di fibroblasti dal Prof. Carlo Manzoni, presso il Policlinico Gemelli. E'

stata preferita la strada più complessa per lo sperimentatore, ma meno traumatica per il bambino di usare fibroblasti anziché mioblasti. Per avere questi ultimi si sarebbe dovuto procedere a biopsie muscolari con necessità di anestetizzare e incidere la cute del bambino, mentre i fibroblasti possono essere ottenuti con un semplice ago-aspirato.

Costruzione di RNA antisenso chimerici

L'impiego dello snRNA U1 per veicolare sequenze di RNA terapeutici dirette contro i siti di splicing di un pre-mRNA è particolarmente indicato poichè, essendo questa molecola normalmente coinvolta nello splicing, consente di colocalizzare il pre-mRNA della distrofina con la sequenza antisenso; il risultato di tale colocalizzazione è un enorme potenziamento dell'efficacia terapeutica della molecola. Lo snRNA U1 è coinvolto specificamente nel riconoscimento della giunzione di splicing al 5'. U1 media tale funzione attraverso l'appaiamento di tale sito con una breve sequenza a singolo filamento presente alla sua estremità 5'. L'interazione U1:5' di splicing verrà in seguito convertita, in un processo di riarrangiamento critico per la corretta definizione di questo sito, nell'interazione U6:5' di splicing. E' stato recentemente dimostrato che, aumentando la lunghezza del duplex formato dallo snRNA U1 e il 5' di splicing, viene inibita la formazione del core cataliticamente attivo dello spliceosoma; in questo modo infatti l'interazione tra U1 e il sito donatore viene stabilizzata impedendo allo snRNA U6 di sostituirsi ad U1. In base a questi dati, al fine di bloccare la reazione di splicing la regione al 5' di U1 sembra essere il sito ideale in cui inserire sequenze antisenso; dati successivi del mio laboratorio hanno inoltre mostrato che l'inibizione contemporanea del sito di splicing al 5' congiuntamente a quella al 3' conferisce un notevole aumento di attività alle molecole antisenso. Pertanto, nel nostro schema progettuale, il gene dello snRNA U1 verrà impiegato per esprimere sequenze antisenso dirette contro i siti di splicing al 5' unitamente al 3'. Inoltre, essendo il promotore di questo gene un polII estremamente forte, otterremo elevati livelli di espressione dell'RNA chimerico. Il razionale che guiderà l'ideazione dei costrutti sarà quello di costruire RNA chimerici che agiscano sul pre-mRNA della distrofina prima che questa venga convertita nella forma matura e trasportata nel citoplasma.

La co-localizzazione del pre-mRNA per la distrofina e lo specifico antisenso portato da U1 snRNA dovrebbero aumentare di molto l'efficacia terapeutica. In genere I primi 8 nucleotidi della regione a singolo filamento di U1 snRNA verranno sostituiti dale sequenze antisenso contro le giunzioni di splicing (o contro le sequenze ESE) di lunghezza variabile tra 45 e 55 nucleotidi. Il mio gruppo ha precedentemente dimostrato che le estensioni anche di 50 nucleotidi non alterano la biosintesi e la stabilità delle molecole chimeriche U1-antisenso. Tutti I cloni saranno ottenuti per PCR inversa sul plasmide phU1-ID, che contiene l'intera unità trascrizionale del gene U1 snRNA. I cloni ottenuti saranno sequenziati al fine di escludere eventuali mutazioni ottenute dall'amplificazione con la polimerasi Taq.

I costrutti ottenuti saranno successivamente clonati nel vettore lentivirale pRRL.sin.PPT.hPGK.EGFP.WPRE, modificato dall'inserzione di un sito Nhe nella regione ΔU3 (vettore pRRL-Nhe): la cassetta antisenso sarà amplificata mediante PCR, dai corrispondenti plasmidi contenenti i costrutti U1, usando primers con estremità NheI. Tutti I prodotti di PCR saranno sequenziati per escludere l'insorgenza di mutazioni.

Preparazione dei mioblasti DMD

I mioblasti saranno isolati da biopsie muscolari; queste saranno pulite da grasso, tessuto connettivo e sangue, divise in piccoli pezzi e messe in capsule di 60-mm di diametro contenenti mezzo RPMI-1640 con aggiunta di 15% FCS, 1% penicillina-streptomicina e 1% glutamina a 37°C in atmosfera CO_2 umidificata al 95%. Le cellule saranno propagate per procedura standard di tripsinizzazione e seminate su piastre rivestite di collagene. Queste cellule primarie muscolari possono essere infettate con vari tipi di vettori e indotte a differenziare per abbassamento di

siero.

In quei casi in cui non si disponga di biopsie saranno utilizzati fibroblasti. Queste cellule potranno essere ugualmente ben analizzate per exon skipping e recupero di sintesi di distrofina se indotte a differenziare a mioblasti mediante trasfezione/trasduzione del gene MyoD.

Preparazione del virus e infezione

L'uso di vettori lentivirali in questa fase del progetto si deve preferire rispetto ad AAV poichè questi virus hanno un'efficienza di trasduzione di cellule muscolari in vitro più alta e la loro produzione è molto più semplice.

Per la produzione di virus sarà utilizzato il sistema a tre plasmidi in cellule 293T. Le particelle virali ottenute saranno ultracentrifugate a 26.000 r.p.m. per 90 min a 4°C. I pellet saranno risospesi in OPTIMEM e conservati a -80°C.

Gli esperimenti di trasduzione saranno compiuti aggiungendo diluizioni seriali di supernatante virale a 2 x 10^5 cellule/pozzetto in presenza di Polybrene (4μg/ml). Le cellule trasdotte saranno poi analizzate per l'espressione della GFP (contenuta nel vettore) mediante citofluorimetria (FACS Calibur, Becton Dickinson Immunocytometry Systems).

I mioblasti (o in alternativa I fibroblasti) saranno piastrati un giorno prima dell'esposizione al virus. 2.5 x 10^5 cellule saranno seminate in piastre da 60 mm. Il giorno dell'infezione, il mezzo di coltura sarà rimpiazzato da supernatante virale con Polybrene. Dopo 12 ore le cellule saranno lavate con PBS e risospese in mezzo di coltura fresco.

Analisi molecolare dell'efficacia dei costrutti antisenso

L'espressione degli RNA chimerici verrà verificata mediante analisi di Northern blot o, alternativamente, per RT-PCR, mentre la presenza di eventuali trascritti che hanno subito skipping verrà verificata mediante nested-RT-PCR impiegando coppie di oligo che consentano di discriminare tali trascritti da quelli in cui non si è avuta correzione. La corretta fusione dei prodotti "skipped" e il ripristino del registro di lettura corretto saranno analizzati per sequenziamento dei prodotti di amplificazione. I prodotti di RT-PCR saranno corsi su gel di agarosio al 2% e le bande specifiche saranno eluite con il kit QIAquick Gel (Qiagen). Il sequenziamento del DNA sarà effettuato utilizzando il BigDye Terminator Cycle Sequencing kit (Applied Biosystems) e analizzato con il sequenziatore ABI Prism 377. Analisi di Western saranno compiute con anticorpi anti distrofina (DYS1 - Novocastra laboratories).

A conclusione di questi esperimenti dovremmo essere in grado di selezionare tra i diversi costrutti prodotti, quello che induca il più alto livello di exon skipping e un più efficace ripristino di sintesi della proteina distrofina.

2) attività relativa al "research agreement" con AMT

L'attività prevista dal research agreement e che si svolgerà in tre anni prevede la costruzione e analisi di costrutti antisenso in grado di dare skipping di esoni, diversi dal 51, che possano "curare" il numero maggiore possibile di mutazioni DMD nell'uomo.

Scelta delle mutazioni DMD

La selezione delle mutazioni su cui intendiamo provare la nostra strategia verrà fatta grazie alla collaborazione con il Parent Project. Quest'associazione dispone infatti di un registro di malati che coprono un'ampia collezione di mutazioni che generano DMD. Molte di queste mutazioni possono essere corrette escludendo dai trascritti singoli esoni. L'obiettivo di questo progetto è

quello di estendere la strategia degli antisenso ad altre mutazioni oltre quella già precedentemente descritta riguardante l'esone 51. In tabella 1 sono riportati gli esoni che saranno oggetto di studio, elencati secondo la percentuale di incidenza nella popolazione. Essendo disponibili pazienti per tutte queste diverse mutazioni procederemo alla costruzione delle molecole antisenso terapeutiche seguendo l'ordine riportato in tabella. Una delle mutazioni meglio caratterizzate è la delezione dell'esone 44; questa delezione, ad elevata incidenza, determina la produzione di un trascritto fuori fase. In questo caso, il ripristino della corretta fase di lettura può essere ottenuto mediante l'ulteriore esclusione dell'esone 45 dal trascritto, con conseguente fusione degli esoni 43 e 46. Lo skipping di questo esone potrebbe "curare" circa l'11 % dei casi DMD noti. Nel caso invece dell'esclusione degli esoni 44 e 53 si potrebbe avere curare un ulteriore 15% della popolazione DMD. In tutti i casi riportati in tabella, da un punto di vista funzionale, le porzioni di distrofina eliminate non dovrebbero abolire la funzionalità della proteina. Infatti distrofine del tutto equivalenti vengono prodotte da individui affetti da una miopatia più lieve della DMD e a volte addirittura asintomatica, nota come Distrofia Muscolare di Becker.

Tabella 1

	Exon to be skipped	**% of deletions in LDMD database**	**Patient code**
1	**51**	**17,5**	**89622, 547/06,494/05**
2	**45**	**11,2**	**290/05, 745/05**
3	**44**	**7,8**	**304/06**
4	**53**	**7,5**	**494/05,523/05**
5	**46**	**5,6**	**1702/05**
6	**50**	**5,2**	**725/05, 1033/05**
7	**8**	**4,5**	
8	**52**	**4**	**725/05, 1033/05;1379/05**
9	**43**	**3,7**	**1616/06**
10	**2**	**2,9**	
11	**17**	**2,8**	
12	**55**	**2,8**	**492/05**
13	**39**		**335**

Analisi dell'efficacia dei costrutti antisenso

Per lo skipping di ogni specifico esone saranno costruiti perlomeno tre diversi costrutti antisenso, seguendo la strategia già definita per lo skipping dell'esone 51. Per ognuno di questi si procederà alla costruzione ed analisi secondo le metodiche descritte più in dettaglio nel paragrafo precedente:

1) Clonaggio di oligonucleotidi (lunghi tra 40 e 55 nucleotidi) contenenti le sequenze antisenso nel vettore pHU1. Il clonaggio in questo vettore permette la produzione di un RNA U1 chimerico contenente nella porzione 5' la sequenza antisenso.
2) Verifica della sequenza dei diversi costrutti.
3) Analisi dei livelli di espressione dei vari costrutti U1-antisenso e della loro stabilità mediante trasfezione in cellule HeLa e analisi degli RNA mediante Northern blot.
4) Se i costrutti prodotti soddisfano ambedue le caratteristiche di alti livelli di espressione e stabilità, questi saranno clonati in vettori lentivirali per ottenere virus che efficientemente infettino cellule muscolari o loro progenitori.
5) Ottenuti i costrutti in vettori lentivirali, questi saranno trasfettati in cellule packaging appropriate e saranno ottenuti lentivirus di cui sarà valutato il titolo in base a test di infettività.
6) I lentivirus ottenuti saranno utilizzati per infettare mioblasti o fibroblasti derivati da pazienti DMD. Nel caso dei fibroblsti, dopo un giorno dalla prima infezione, essi saranno superinfettati con un retrovirus contenente il gene MyoD che induce differenziamento muscolare.
7) Dopo 10 giorni dall'infezione le cellule verranno raccolte e saranno estratti sia gli acidi nucleici che le proteine.
8) L'espressione delle molecole antisenso così come lo skipping dei diversi esoni saranno quantificati su RNA totale mediante RT-PCR.
9) In parallelo, il recupero di sintesi di distrofina sarà quantizzato mediante analisi di Western blot.
10) Il diverso grado di efficienza nell'indurre exon skipping e recupero di distrofina sarà valutato normalizzando questi parametri per l'efficienza di infezione dei lentivirus.
11) Come ultimo punto sarà importante verificare se le diverse molecole di antisenso in oggetto siano efficaci nelle cellule ottenute da pazienti DMD con mutazioni diverse tra loro, ma tutte curabili con lo skipping di uno specifico esone.

Nell'arco dei tre anni del progetto preventiviamo di progettare, costruire e analizzare per attività almeno 12 costrutti per almeno 4 diversi skipping. I costrutti più attivi saranno ceduti ad AMT.

Analisi della distribuzione sistemica di virus AAV di sierotipo di tipo 6

La AMT possiede la tecnologia per la produzione ed uso di vettori AAV di sierotipo 6. Poiché le nostre analisi precedenti hanno mostrato l'efficace trasduzione in vivo di tutti i distretti muscolari, compreso cuore e diaframma, con AAV di sierotipo 1 e 9, parte dell'attività da svolgere nell'ambito del research agreement con AMT sarà dedicata alla verifica della capacità di traduzione in vivo di vettori AAV6. A tale scopo particelle virali, contenenti la cassetta di espressione dell'U1 antisenso, insieme al marcatore GFP, saranno iniettati a concentrazioni scalari (10^{12}, 10^{11} e 10^{10}) nella vena caudale di animali mdx. A intervalli di tempo di 1 e 3 mesi gli animali saranno sacrificati e sarà analizzato il livello di colonizzazione dei vari distretti muscolari mediante analisi della GFP. In parallelo, sarà analizzata l'attività di exon skipping e il recupero della distrofina. Se i valori di trasduzione e "cura" saranno simili a quelli ottenuti precedentemente con i sierotipi 1 e 9, si procederà all'uso di AAV6 per gli studi tossicologici e pre-clinici.