

THE ESACT NEWSLETTER

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Editorial **Gene therapy and adverse events**

In April this year the American Society of Gene Therapy (ASGT) instituted its policy that "all investigators and team members responsible for patient selection, the informed-consent process, and/or clinical management in a trial must not have equity, stock options or comparable arrangements in companies sponsoring the trial".

ASGT's aim in ensuring that clinical research studies are carried out "in an objective and unbiased manner" is surely praiseworthy but it should not have been necessary to say so. The move may not have been caused by reports of the suppression of "adverse event" reporting in gene therapy trials, but ASGT's fortuitous policy clarification followed closely a stern reminder from NIH.

Prior to this only 39 adverse events had been reported from 93 gene therapy studies. The rap on the knuckles from NIH resulted in a further 650 events from being reported from the same 93 studies. Theoretically negative results in clinical trials should be as valuable as positive ones. Sadly this is not the way shareholders or company directors see the situation. There have been too many cases recently of biotechnology companies unjustifiably pushing high-profile products into further clinical trials. Compared to mainstream pharmaceutical groups, biotechnology companies proportionately put more of their compounds into phase II and phase III trials and proportionately more fall by the wayside.

Unwarranted optimism is both mendacious and expensive. Unfulfilled medical needs remain unmet, investors money is wasted and investigative time is squandered. Goal-directed research can be a positive aid to efficiency but bias in the interpretation of results, however unintentional, is a bad thing.

The Editor

From our Chairman: Manuel Carrondo

Thank heavens I am no longer running for re-election as ESACT's Chairman. Indeed, this is a hard time to address ESACT's members, either going to, or returning from their holidays. Thus I can afford to write a light editorial which can go through with a sip from your refreshing cocktail.

I will leave the attempts to bridge our activities to clinical practitioners and the results from the workshop entitled 'From Medical Biotechnology to Clinical Practice', under the aegis of the EU, for the next issue as well as our ongoing activities with ACTIP, EFB, and ESGT (European Society for Gene Therapy) (NB at their next meeting in Stockholm, 7-10th October 2000, registration fees are the same as for ESGT members). I will concentrate on just two topics: Meetings and the next Executive Committee.

ESACT's **17th Meeting in Tylosand, Sweden (June 10-14th 2001)** is in full gear. The confirmed invited speakers are great, as we are now used to, the sessions have good Chairpersons, and the social events will be a delight - we expect nothing less of Elisabeth Lindner-Olsson. You will remember she was going through the Pharmacia and Upjohn merger when she took on this commitment, and now is organising the meeting whilst the new merger with Monsanto is ongoing. You will appreciate how reliable, hardworking and enjoyable your Meeting Secretary is! I am sure you will all join me in wishing Elisabeth all the best both for her career negotiations during the merger and for her success in organising our meeting. Soon you will get the final announcement, so please start getting ready to submit your Abstracts in order to improve the quality and novelty of the ESACT science.

Meanwhile the Executive Committee has approved the choice proposed by Francesc Godia for **ESACT's 18th meeting in mid-May 2003**. This will be in the beautiful town of Granada, in southern Spain, renowned for the Alhambra - one of the best pearls from the time when the Moors had the most elaborate art and novel architecture in Europe. The town centre is full of small squares and nice houses, so you will find the whole place cosy as required by ESACT's standards.

Thus the time is ripe for the meeting secretary for the 19th Meeting to come forward for 2005.

Coming all the way from Sweden in 2001 to Spain in 2003, where shall we go next? East Central Europe (Hungary, Czech Republic..?), South East (Greece..?) or where..? This brings me to the discussion on the composition of the next Executive Committee. Over the past few years we have been able to elect to the committee the Meeting Secretary for the next but one Meeting. This allows an 'educational' period that provides for smoother organisation of meetings which are the main activity of ESACT and the gathering point for our members. Since in Sweden next June the next executive committee will be elected I would urge the potential meeting secretary(-ries) for 2005 to come forward with their proposals for consideration at the next Committee Meeting in February 2001. At the same time please prepare yourselves to nominate or to be nominated for election - ESACT needs new blood. As in the recent past we would like to see at least 2 of the 8 Committee positions being taken by new members.

Enjoy your holidays and keep contributing to ESACT's continuous advancement.

Manuel Carrondo

New technologies/ new ideas/ newconcepts

The ESACT *Newsletter* was restarted several years ago in order to serve the members of ESACT. Although it functions as such for the moment, the ESACT Executive Committee did not want to create a medium announcing its own views, but the idea of restarting the ESACT *Newsletter* was also to provide a means for interaction between the ESACT-members and to provide ESACT-members the opportunity to put forward their views etc.. For instance ESACT *Newsletters* which were for a certain time 15 years ago, presented several controversial views on different aspects of animal technology. In order to revive the possibility to exchange controversial views, to present new technologies/new ideas/new concepts we have decided to start this information section on new technologies/new ideas/new concepts.

The first presentation concerns information on fluorescent cells by Prof. Florian Wurm from the Swiss Federal Institute of Technology, Lausanne (EPFL)/Ch. These might find some general use in mammalian cell culture and development.

Otto-Wilhelm Merten

Fluorescent mammalian cells

An essential parameter in research and development work with animal cells is the determination of the number of viable cells in culture. Standard methods rely on manual (microscopic) or instrument-assisted cell counting, requiring sampling and/or dilution of cells. We propose an alternative that is much simpler and very precise.

Using commercially available DNA sequences and vectors coding for highly fluorescent proteins (enhanced green fluorescent protein, yellow fluorescent, cyan fluorescent protein) one can modify mammalian cells so that they emit a strong fluorescent light signal when excited. A variety of stable Chinese hamster ovary cell lines (CHO) and Human embryo kidney cell lines (HEK-293), recently established, have been studied in detail. The proteins expressed so far are designed to remain in the cytoplasm of the cells. It was shown that the emitted fluorescent signal is a reliable and constant character of each of the cell lines under study, permitting its use for the determination of viable cell mass.

Because of the ease, speed, reliability and low error rate of standard methods available for the quantification of fluorescent signals (for example, with the help of standard fluorescent multiwell-readers, adaptable to formats from the 6 well to the 386 well format) reproducible data can be obtained from a large number of individual cell cultures, simultaneously and/or within very brief time intervals. We have done repeated fluorescent readings of cells in multiwell tissue culture plates in time intervals as short as 2 hours. These measurements reflected growth and/or arrest of the concerned cell populations with a precision sufficient for growth rate determinations. With classical techniques such calculations require measurements taken over days and that also would have higher error rates.

We are applying this technology for the rapid assessment of environmental conditions in the context of process development for recombinant protein production from batch cultures and for applications in a tissue-engineering project. We have found that the co-expression of fluorescent protein together with the protein of interest is also a useful tool for the identification of highly productive cell lines.

Due to the very high expression rate in some of our cell lines very intense fluorescent signals can be obtained. Using a fluorescent microscope, a single cell can be detected in over-confluent adherent or high density suspension cell populations.

We are presently looking for potential applications of these cell lines or similar ones we could generate, both in terms of an industrial collaboration and/or an academic research program.

For further information please contact me. Details are under ESACT information on the last page of the *Newsletter*

Florian Wurm

The 7th Meeting of the EUROPEAN SOCIETY OF GENE THERAPY - MUNICH, NOVEMBER 26-28 1999

ESACT held its joint meeting with European Society of Gene Therapy formerly known as the European Working Group on Human Gene Transfer and Therapy (EWGT) in Munich last November. The meeting was extremely well attended with over 500 clinicians, molecular biologists, virologists and animal cell technologists coming together at the Technical University of Munich to hear a wide variety of high quality presentations.

The programme opened with three symposium sessions on viral vectors.

The first of these covered lentiviral vectors and opened with a presentation by Naldini from Turin describing the strategies that their group have adopted to develop vectors with improved transduction frequencies, enhanced transgene expression and increased safety. Subsequent presentations by Russell (Rochester, USA) and Cichutek (Langen) both addressed the issue of how to target lentiviruses to particular cell types. The final paper in this session by Cosset (Lyon) described experiments that they have undertaken to develop lentiviral vectors which are capable of infecting primary T4 lymphocytes.

The second and third symposia contained presentations relating recent developments with other types of viral vectors. These included

retroviral vectors (Collins, London), adeno-associated viral vectors (Hallek, Munich and Joos, Evry), adenoviral vectors (Kochanek, Cologne and Vigne, Villejuif) and cytomegalovirus (Messerie, Munich) - a promising new candidate for gene transfer.

The fourth session was devoted to non-viral vectors and included presentations describing new ways of targeting DNA complexes using transferrin complexes (Wagner, Vienna), of introducing vectors with high efficiency by using electrical pulses (Scherman, Vitry-sur-Seine) and a presentation by Behr on overcoming barriers to nonviral gene delivery.

A simultaneous session was held addressing recent developments in the gene therapy of cardiovascular disorders including restenosis, atherosclerosis, angiogenesis and ischaemic disease.

On Saturday morning we were spoiled for choice with three simultaneous sessions; one devoted to cancer, one to neuromuscular disorders and a third to eugenethy. After coffee the choice continued and the ESACT session was on at the same time as two offered paper sessions. There were five papers in the ESACT session, the first of these given by Morrey Atkinson of the Targeted Genetics Corporation in Seattle. He described the industrial-scale GMP production of adeno-associated viral vectors using a stable cell line in a stirred-tank suspension bioreactor.

The vectors were being developed for the treatment of cystic fibrosis and there was a need for high doses. Although it is possible to get high levels of vector produced from adherent cell cultures using cell factories, typical levels of expression in suspension culture are low (of the order of 100 particles per cell). Atkinson described experiments in which he optimised the system and was able to produce yields of 50 000 particles per cell in bioreactors up to the 10m scale.

The second paper by Kieny from Transgene, described their experiences in using an attenuated recombinant vaccinia virus for the immunol gene therapy of cervical cancer. The approach taken was to vaccinate against the transforming proteins E6 and E7 of human papillomaviruses types 16 and 18. Recombinant non-replicative vaccinia viruses were constructed which contained modified E6 and E7 genes as well as human IL2 and these have been shown to be capable of resulting in rejection of

tumours developed in a mouse model.

The paper by Otto Merten from Généthon, described the yields obtained from different bioreactor systems when used to produce retroviral vectors. The systems used were the Cellcube, a Celligen fixed bed reactor, a Celligen cell lift reactor and clump culture in stirred-tank reactors. The highest titers were obtained from the Cellcube and the fixed bed reactor however neither of these systems were particularly suitable for scale-up. The microcarrier system, in contrast was easy to scale-up, easy to monitor growth and was based on a known technology, but had the disadvantage of slower growth rates resulting in lower cell numbers and a lower titer of virus. The old biotechnology adage springs to mind "horses for courses".

Caroline MacDonald

The 7th Cell Culture Engineering Meeting, Santa Fe, New Mexico February 5-10, 2000

Of almost 240 participants at this meeting, more than 70 per cent were from industry with most of the remainder from academia.

The programme which consisted of 8 oral sessions as well as workshops and poster presentations, began with virus and vector production in cell culture for vaccines and gene therapy. Three lectures dealt with the modifications and improvement of adenoviral by Wickham (GenVec, Rockville), Massie (Inst. Rech. Biotech, Montreal) and Leopold (Cornell Univ. New York) and Otto Merton (Généthon) compared different reactor systems for the production of retroviral vectors.

The second session on cell production for cell and gene therapy started with a presentation by Forestell (Systemix, Palo Alto) on using ProPak A packaging cell lines for the production of retroviral vectors. Peshwa (Dendreon, Seattle) presented a process for preparing dendritic cells for immunotherapy while Papontsakakis and Carswell (Northwestern Univ., Evanston) described the optimisation of conditions for T cell population expansion in immunotherapy.

A third session on cultured cells and tissues for transplantation and as models commenced with a presentation from Good (Texas A & M Univ) on in vitro models for head injury and glaucoma

followed by DeMilla (Organogenesis, Canton) who described a new hepatocyte culture device and Bonassar (Univ. Mass. Medical School) on chondrocyte culture for cartilage repair.

The fourth session on cell physiology and metabolism was opened by Lauffenburger (MIT, Cambridge) with a lecture on autocrine ligands and cell functional response in culture. Häggström (Royal Inst. Tech., Stockholm) presented the metabolic diversity of Sf9 insect cells and the effects of autocrine factors on Sf9 cell growth, while Sundström (Univ. New South Wales) described the role of IGF-1 on growth and survival of CHO cells. Balcarcel and Stephanopoulos (Vanderbilt Univ. Nashville) lectured on the role of metabolism in apoptotic cell death in mammalian cellculture.

Session five on protein quality/glycosylation was opened by Jacobson (Genentech), Riggin and Huang (both from Eli Lilly) discussing the quality control of glycosylated proteins and analytical methods used. Schlokot (Baxter Healthcare, Orth/Donau) described the use of an endopeptidase, shed furin, in the maturation of complex glycoproteins while Jäger (GBF, Braunschweig) discussed the intracellular accumulation of rec. proteins in baculovirus infected insect cells.

In the sixth session on cell and metabolic engineering, Fussenegger (ETH, Zurich) discussed new compatible gene regulation systems responsive to streptogramin antibiotics, Betenbaugh (John Hopkins, Baltimore) described insect cell engineering for correct glycosylation of rec. proteins and Ryell (Genentech) presented the modification of protein glycosylation in CHO cells by using transferases or by modifying the culture medium. James (Univ of Kent, Canterbury) described mRNA translation initiation in CHO cells.

Session seven dealt with high throughput gene, expression and was opened by Jordan & Wurm (EPFL, Lausanne) who described the optimisation of Ca-phosphate transfection using 293-EBNA cells. Gray (Univ. New South Wales) presented a new rapid selection system for high expressing CHO clones while Bachmann (Cytos Biotech, Zurich) presented the DELphi technique.

In the final session on manufacturing issues Deo (Medarex, Annandale) presented a new nude mouse - based hybridoma system, Black & Ramakrishnan

(Lilly Res. Labs, Indianapolis) described scale-down systems for late stage process development, Sherwood (Lonza Biologics, Slough) presented Lonza's contract production capabilities, Chang (BASF Bioreseach, Worcester) described the BASF platform for human Mab production using rCHO cells and Moran (Glaxo Wellcome R&D, Beckenham) discussed bioprocess validation in Mab production in murine myeloma cells.

Otto-Wilhelm Merten

ESACT 17 June 10-14 2001 Tylösand, Sweden 'From Target to Market'

The meeting is advancing in preparation and we plan to publish the 2nd Announcement in early September. Sessions include:

1. Identification of Drugs and Drug Targets
Chairs: Karin Mellström, KaroBio and Johann Häggblad, Pharmacia
2. Expression Systems for Target and Drug Production
Chairs: Peter Liljeström, Karolinska and Alain Bernard, Serono
3. Cell Physiology and Metabolism
Chairs: Lena Häggström, Royal Institute of Technology and Roland Wagner, GBF
4. Production Technology
Chairs: Leif Kongerslev, Novo Nordisk and Christel Fenge, AstraZeneca
5. Changes in the Regulatory Environment
Chairs: Sarbari Roy, BioInvent and Erik Thorsell, MPA Sweden
6. Novel Technologies for Administration of Cell Derived Proteins
Chairs: Elisabeth Lindner-Olsson, Pharmacia and Daan Crommelin, University of Utrecht
7. Novel Prophylactic and Therapeutic Approaches based on Animal Cells or Nucleic Acids
Chairs: Hansjörg Hauser, GBF and Otto-Wilhelm Merten, Généthon

8. Biotech in the New Millenium Interactive session

We have today confirmed the following KeyNote speakers:

Lennart Philipsson, Karolinska
Bengt Westermark, Uppsala
Lawrence Chasin, Columbia Univ.
Marina Cavazzana-Calvo, Hôpital Necker

This panel together with about 10 already confirmed invited speakers gives us great hope for a good and fruitful meeting in Tylösand.

Elisabeth Lindner-Olsson

JAACT 2000 Fukuoka: November 17-21, 2000

The second circular for JAACT 2000 is now available on the JAACT website (<http://www.agr.kyushu-u.ac.jp/jaact/>) You will find many interesting topics in the plenary lectures, educational lectures and 9 symposia, and in the Karatsu satellite symposium. In addition to the scientific programme, excursions will include a visit to Karatsu Castle, Sumo wrestling, a historical tour in the Fukuoka region and a Super-Kabuki traditional theatre visit.

The deadline for registration has been extended to July 31, 2000. The organising committee welcomes all ESACT members to JAACT 2000.

Sanetaka Shirahatu
Chairman of JAACT 2000

Note: Details of JAACT 2000 can also be reached through the MEETINGS or LINKS sections on the ESACT website: www.esact.org

Other Meetings

- August 26-30 Vienna, Austria
Pharmacy World
Congress 2000
- Sept. 3-8 Berlin, Germany
Biotechnology 2000
- Sept. 11-13 Bethesda, Maryland
In situ hybridization
- Sept. 17-18 Amsterdam, Netherlands
European Biotechnology
Symposium
- Sept. 18-20 Paris, France

- 2nd World Vaccine
Congress
- Oct. 5-8 Semmering, Austria
Recombinant protein
production with
prokaryotic and eukaryotic
cells
- Oct. 12-13 Paris, France
Pharmacogenetics and
pharmacogenomics
- Oct. 16-17 London, England
8th Biopartnering Europe
- Oct. 30-Nov.2 Basel, Switzerland
Laboratory automation and
robotics
- Nov. 13-15 Berlin, Germany
Bio-Europe 2000
- Dec. 4-6 Ottawa, Canada
The Bioproducts
revolution

European Commission News

June saw the launch of the new BioSociety web site. It will serve as a platform to collect and disseminate information on socio-economic research in the life sciences and as a forum to stimulate thinking on the emerging biosociety.

Contact: <http://biosociety.dms.it>

BioBiz: preparing a business plan. This is a series of 3-day training workshops being held across Europe up to November 2000.

Contact: michel_lepers@compuserve.com

The second deadline for the second call for proposals for funding is 11 October 2000. Specific sub-areas under the activity heading "The cell factory" include 3.1.1, 3.1.3, 3.2.2, 3.2.4, 3.2.5 and 3.3.4. Documents needed to prepare and submit a proposal are available on the Web: www.cordis.lu/life. Questions on the programme should be addressed to:

quality-of-life@eec.eu.int or by fax: +322.299.1860

New Members

Since our January 2000 Millenium *Newsletter* we are happy to welcome the following new members; Michael Brailsford, Chemunex, Ivry sur Seine; Christoph Geserick, Schering, Berlin; Matilde Guembes, Laboratorios Bruch, San Paulo; Joanna Harrison, University College, London; Henry

Hoppe, Genzyme Corporation, Framingham; Meinhart Horst, Foerdergesellschaft Chemnitz, Heidelberg; Chang-Chun Hsiao, Mingchi Institute of Technology, Taipei; Toni Lindl, Institute of Applied Cell Culture, Munich; Lars Soegaard Nielsen, Novo Nordisk, Bagsvaerd; Lottie Norrsen, Active Biotech Research, Lund; Andrew O'Toole, Nova Biomedical, Warrington; Antonio Pires, Elnor, Maia; Markus Rohde, Homberg/Efze; Annelie Sjoberg, Active Biotech, Lund; Daniel Stark, EPFL, Ecublens; Helmut Trautmann, Biospectra, Zurich; John van der Veecken, JM Separations, Ridderkerk; Steffen Zeng, Biochemie, Kundl.

ESACT eNEWS

- The eNews is intended to supplement the *Newsletter* by keeping members informed of news and events between *Newsletter* publication times
- You can all make use of the eNews to notify members of meetings/events/news etc. by sending your announcement to Christophe Losberger for inclusion in the next edition
- The frequency will depend entirely on the quantity, quality and urgency of the news we have
- We will keep you updated on the organisation of future meetings, and other subjects could include European Commission initiatives, news from organisations that we collaborate with (ACTIP, EFB, JACC, National Societies etc.), and relevant Courses, Publications, and Industrial developments
- If you do not wish to receive these Bulletins then please inform Christophe Losberger (Contact details on the back page)

Bryan Griffiths

ESACT SECRETARIAT

Firstly my usual plea for members to keep me informed of their address details - I enclose a form for this purpose (or use the website). **There are many Email addresses either missing or wrong**

so I particularly want to update this part of the database as we will increasingly be using this system to communicate with members (eg the ESACT eNEWS launched in March 2000). Also those of you who no longer wish to be members of ESACT please let me know as I spend a considerable amount of time and resources chasing up lapsed members.

Secondly a plea for **Subscriptions** - these are now well overdue for 2000. Payment details are also enclosed with this *Newsletter*. American Express has now been added to the credit/debit card list we can process. Also the website is being upgraded to allow secure payments by this method. Members who have not paid since 1997 will be automatically deleted from the membership list unless they contact me.

Thirdly a reminder that copies of the **Proceedings** of the last 3 meetings are available from the ESACT Office at special rates to members;

- 14th Meeting (Vilamoura) Price £20 including postage
- 15th Meeting (Tours) Price £20 plus postage
- 16th Meeting (Lugano) Price £50 plus postage

Fourthly as we are continuing to print the cover page of the *Newsletter* (rather than photocopy) we can now include photographs. So anyone with an interesting Photograph (especially with a short report) please send into the *Newsletter* editor. Also as the contents of the *Newsletter* are largely committee and editor generated we would like to see some articles, information, news or any contribution from members - there are over 350 of you so you must have information to impart of real interest to us all

Finally a reminder to use the ESACT website (www.esact.org). The webmaster (Christophe Losberger) is enthusiastically doing a great job of regularly updating the web pages with information on meetings, publications, jobs and activities of Societies with overlapping interests.

With best wishes to everyone

Bryan Griffiths
ESACT OFFICE

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Membership Subscriptions:

Full Members - £13 (Standing Order to ESACT Bank Acct.£11)
 Associate Members - \$25

Note:

- Preferred payment method - Credit (not Charge) Card
- We recommend you consider payment of more than one year's subscription at a time so as to minimise local bank charges to your account.
- If you wish to pay by standing order, payment instructions should be for account ESACT, A/C 01054392, National Westminster Bank, Salisbury (Sort Code 54-41-19) with your payment instructions endorsed "charges for payers account".

Newsletter Correspondence/Contributions:

Correspondence for publication in the ESACT Newsletter, meeting reports and comments should be sent direct to the Newsletter Editor.