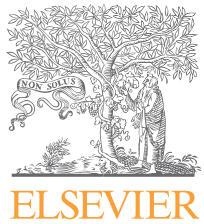
EJC SUPPLEMENTS 10, NO. 1 (2012) 128–140



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EORTC-related new drug discovery and development activities: role of the Pharmacology and Molecular Mechanisms Group

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ARTICLE INFO

Keywords:
Cancer
Pharmacology
Molecular mechanisms

ABSTRACT

The EORTC Pharmacology and Molecular Mechanism Group (PAMM) focuses on applied research to translate basic/fundamental research discoveries in cancer biology into new drug discovery and development. PAMM provides a unique platform on the pharmacology, pharmacokinetics, pharmacodynamics of drug effects, molecular mechanisms of anticancer agents, and drug-related molecular pathology. For these purposes the group stimulates the interaction between basic scientists and clinicians in order to perform translational research on the pharmacology and molecular mechanisms of anticancer agents in Europe. The group has extensive expertise in various disciplines of pharmacology and has developed standards for studies performed in conjunction with clinical trials equivalent to those of good laboratory practice (GLP). The group serves as master organization for other EORTC (sub-)committees in the maximal interest of these groups and of the EORTC as a whole. PAMM merged with Preclinical Therapeutics Models Group (PTMG) in 2000 and with the Screening and Pharmacology Group (SPG) in 2003. The latter group continued as the Drug Discovery Committee within PAMM. The groups have always been involved in the development of anticancer agents, evolving from platinum analogs, anthracyclines, nitrosoureas, antifolates in the 1980's, to drugs derived from natural sources (trabectedin, taxanes) in the 1990's, and anti-signaling drugs, DNA alkylators, in the last decade. Several of these drugs have been registered. Mechanistic studies focused on drug activation/inactivation, target (DNA, receptors) in relation to efficacy and toxicity such as with several antimetabolites (5-fluorouracil, methotrexate), topoisomerase inhibitors (irinotecan), tyrosine kinase inhibitors (imatinib), acridones (C-1311), etc. The group recently included pharmacogenetics in the identification of genetic polymorphisms in order to use this information for personalized therapy.

1. Introduction

The EORTC Pharmacology and Molecular Mechanism Group (PAMM) was established as the EORTC Pharmacokinetics and Metabolism (PAM) project group in early 1978 and had its first formal meeting in December 1978 in Cambridge, UK. The meeting was initiated by a small group of oncologists and pharmacologists who

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Table 1 – List of EORTC PAMM, SPG and PTMG officers

Year	EORTC PAM		EORTC SPG	EORTC CASG	NCI cpds
	Chair	Secretary	Chair	Chair	Chair
1972			L.M. van Putten, Rijswijk		
1978	J.F. Smyth, Edinburgh	H.M. Pinedo, Amsterdam	F. Spreafico, Milan		
1979		W.J.F. van der Vijgh, Amsterdam			
1982	K. Harrap, Sutton	J.G. McVie, Amsterdam	B.W. Fox, Manchester	M. Aapro, Genolier	
1985	J.G McVie, Amsterdam	P. Workman, Cambridge			
1987			M.G.M. Stevens, Birmingham		
1988	P. Workman, Cambridge	M. D'Incalci, Milan			
1990			J.A. Double, Bradford		
1991	M. D'Incalci, Milan	D.R. Newell, Newcastle			
1994	D.R. Newell, Newcastle	J.H. Schornagel, Amsterdam		C. Dittrich, Vienna	
1995					D.R. Newell, Newcastle
1997	J.H. Schornagel, Amsterdam	A. Gescher, Leicester			
2000	A. Gescher, Leicester	J.H.M. Schellens, Amsterdam	I. Fichtner, Berlin	J.L. Merlin, Nancy	J. Double, Bradford
2003	J.H.M. Schellens, Amsterdam	N. Zaffaroni, Milan	M. Bibby, Bradford		I. Fichtner, Berlin
2006	N. Zaffaroni, Milan	F. Gieseler, Kiel	A.M. Burger, Detroit		A.M. Burger, Detroit
2008					G.J. Peters, Amsterdam
2009	G.J. Peters, Amsterdam	E. Chatelut, Toulouse			
2011					A. Westwell, Cardiff

cpds, compounds.
Treasurers of the PAMM: A. Gouyette (1987–1991); J. Robert (1991–2003); A. Larsen (2003–). J.G. McVie served as EORTC president (1994–1997); the late Tom Connors (1991–1997), M. D'Incalci (1997–2000) and D.R. (Herbie) Newell (2000–2003) served as chair of the Laboratory Division; P. Workman (1991–1993) and M. D'Incalci (1994–1997) as chair of the New Drug Development Committee; J. Double as treasurer of the EORTC. Others served (or still serve) as member of the TRAC, NDAC or on the board of the EORTC. Several members subsequently served as officer of other groups.

felt that there was a lack of interaction in the field of drug development. When the group was established, it formulated three main functions: (1) to provide a forum for investigators working on the clinical pharmacology of anticancer drugs, (2) to explore the potential for cooperation between different European centers working on the pharmacology and metabolism of anticancer drugs, and (3) to provide a link between the EORTC Screening and Pharmacology Group (SPG) and the Early Clinical Trials Group (ECTG). Dr. John Smyth (then at the Institute of Cancer Research [ICR] in Sutton, Surrey) was the first Chair. The first two aims still form the basis for the current PAMM, and regarding the third aim, the EORTC SPG has been part of PAMM as a separate committee, the Drug Discovery Committee (DDC) since 2003. Interaction with the ECTG (since 1995 the ECSG, the Early Clinical Trials Study Group and since 2002 the New Drug Development Group) has now been replaced by interactions with several of the disease-oriented groups with a focus on translational research. The SPG was established in 1972 with the aim to provide facilities for drug screening and its further development. The former Clonogenic Assay Screening Group (CASG) was established in 1981 as a subgroup of the ECTG and initially named the Clonogenic Assay Study Club (CASC), and was renamed in 1993 as the Preclinical Therapeutic Models Group (PTMG). It merged with PAMM in 2000. These mergers were natural processes since the groups usually met at the same time and many activities became joint activities (Table 1, Fig. 1). Naturally the three groups had many achievements in drug development; within the framework of the groups several new chemical entities (NCEs) were developed, while the groups also provided early screening for antitumor activity of NCEs associated with proper preclinical pharmacology and mechanism of action studies, followed by clinical pharmacology studies in close collaboration with pharmaceutical companies and

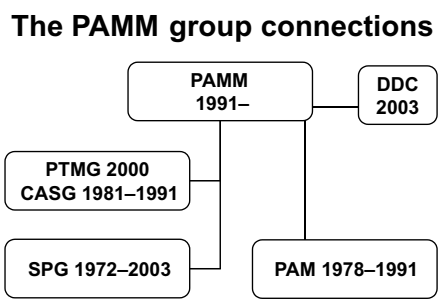

Fig. 1 – PAMM Connections. The short connection (2006–2009) of the imaging group is not shown since this group was re-established as the Imaging Group in 2009.

Table 2 – Special meetings of the EORTC-PAMM and SPG alone or with other organizations

1. First PAM meeting on "Drug interactions in cancer Chemotherapy", December 5–7, 1978, Cambridge, UK
2. Joint BACR, PAM and SPG meeting, March 24–27, 1985, Aston University, Birmingham
3. New concepts in anticancer drug design (SPG), December 10–11, 1986, Rijswijk, The Netherlands.
4. 10th Anniversary meeting of the PAM group; special PAM-SPG: symposium: Chloroethyl nitrosoureas: the beginning of the end or the end of the beginning? December 14–17, 1988, Jesus College, Cambridge, England
5. Special BACR-PAM symposium: The cell membrane and cell signals as targets for novel anticancer drugs. September 14–16, 1989, Queen's College, Cambridge, England
6. Screening for bioreductive anti-cancer agents (PAM-SPG). July 6–7, 1989, Bradford, England
7. Target-specific agents: antisense oligonucleotides, new DNA cleaving molecules and immunomodulatory molecules (SPG). December 7–8, 1989, Toulouse, France
8. Contribution to the International Symposium on Immunodeficient mice in oncology (SPG). November 7–9, 1990, Freiburg, Germany
9. Novel approaches in cancer therapy, (PAMM, SPG and AWO/Phase I/II study groups). December 1–4, 1993, German Cancer Research Center, Heidelberg, Germany
10. Acridine derivatives as potential antitumor agents (SPG). June 17, 1994, Manchester, England
11. Standard Operating Procedures (SPG). January 21,1998, Nancy, France
12. 20th Anniversary Meeting of PAMM, PAMMsterdam, January 20–23, 1999, Amsterdam, The Netherlands
13. Special PAMM, SPG symposium on In Vivo models, January 31 – February 3, 2001, Verona, Italy.
14. First PAMM Functional Imaging Joint symposium, January 26–29, 2005, Arcachon, France
15. 30th Anniversary Meeting; a special EGAM-PAMM symposium. March 17–18, 2009, Brussels, Belgium

the ECTG.[1] Many of these NCEs are now registered as an anticancer drug and form an important and essential constituent of treatment regimens for many cancer types. Several of these achievements are discussed in the following sections.

Officers of the three groups, the PAM, SPG and CASG, always contributed intensively to the EORTC, both as a scientific contribution to early preclinical and clinical drug development, and by supporting the EORTC. Several chairs and officers of the groups have served on the EORTC Board either as member, but also as EORTC president, treasurer, or otherwise such as head of the Laboratory Division (Table 1). Members of the groups also initiated many international activities; several PAM and PAMM meetings were jointly organized with national organizations as well as with other European and American groups. Recently collaborations with other continents were established as well (Table 2). Hence the three groups served (and the current PAMM as well) as an excellent basis for many collaborations advancing science, cancer research and promoting the careers of many young scientists. PAMM meetings are characterized by open discussion and care is taken that young scientists get the chance to present their data for a critical but friendly audience. Hence, each meeting is concluded by giving an award to the best young investigator presentation.

2. The EORTC Screening and Pharmacology Group (SPG)

The SPG is one of the oldest EORTC groups (Table 1). It was established in 1972 out of a merger between the Experimental Screening Group and the Preclinical Pharmacology Group. In 2003 the group merged with PAMM, and its work was continued as the DDC. The main objective of the SPG was to provide antitumor and toxicity testing systems to investigate new agents and study their mode of action, toxicity, and other pharmacological properties. In this way the group may alert scientists (within the EORTC) to NCEs that are potentially interesting in the treatment of cancer. For this purpose drug dossiers were made available. The SPG and now the DDC has members in the major cancer research institutes of Europe. The membership is a balance of chemists, biologists, and clinicians with an interest in many aspects of new drug development such as synthesis, primary screening, secondary evaluation, mode of action, toxicology, etc. This close collaboration between various disciplines facilitates structure/activity studies and analog development to ensure that only agents with a real potential will progress to clinical trials.

All meetings of the SPG and now the DDC are held under a confidentiality agreement, and all members sign a statement declaring that results of the examined compounds will not be discussed or disclosed outside the membership of the group. The business meetings are typically round-table discussions on chemistry, screening results, pharmacology, and toxicology for a range of different compounds. The aims of the meetings are to collate information from the various laboratories, identify areas where further work is needed, and produce dossiers on new drugs for submission to the New Treatment Committee for possible progression to clinical trials. The nature of the drug dossiers has changed with time and included 1,2:5,6 diepoxyhexane (1982), disuccinyl-dianhydrogalactitol (DiSu-DAG) (1983),

Table 3 – List of miniprojects supported by the EORTC-SPG and DDC group

1985

1. Synthesis of potential bioalkylating cyclopentane dimethane sulfonates (Reinhoudt).
2. Synthesis of 2-chloroethyl-N-nitrosocarbamoyl (CNC) amino acids and -dipeptides and their methylamides for testing in experimental tumor models (Eisenbrand, Atassi).
3. Comparison of tumor growth inhibitory and toxic effects of BCNU, 5-FU, a combination of BCNU + 5-FU and that of B-3839 (Somfai).

1986

1. Antitumor activity of B compounds against subcutaneous colon 38 carcinoma (Atassi).
2. Activity of N-[N1-(2-chloroethyl)-N1-nitrosocarbamoyl (CNC)]-alanine and derivatives against a panel of transplantable adenocarcinomata of the mouse colon (Double).

1987

1. Synthesis of B.3839 (Reinhoudt).
2. Effects of routes of administration of TCNU on its plasma, tissue and tumor concentrations (Double).
3. The influence of routes of administration of TCNU on antitumor activity in a panel of MAC tumors (Double).

1988

1. In vitro and in vivo antitumor activity of mitomycin C and three mitosene analogues (Verboom, Lelieveld, Fiebig, Double).

1989

1. Synthesis and testing of E2-linked WV14 (Reinhoudt, Eisenbrand).
2. Synthesis and testing of hormone-linked mitosene derivatives (Reinhoudt, Eisenbrandt).
3. Determination of the antitumor activity and bioreductive potential of the mitosene GBJ584 (Double, Lelieveld).
4. Pharmacokinetics of "B compounds"; correlation with anti-tumor activity (Double, McElhinney).

1991

1. In vitro/in vivo antitumor activity of a series of indolo-(2,3-b)quinoxalines (Cros, Double, Lelieveld).
2. Synthesis and testing of some 2-arylbenzothiazoles (Stevens, Double, Lelieveld).
3. Synthesis of seven nitrosoureas with pyrimidin-3-yl substituents for comparison with effective N1 analogues and for DNA cross-linking studies (McElhinney, Double).
4. Novel heterocycles as potential protein tyrosine kinase inhibitors (McElhinney, Stevens, Lelieveld).

2002

1. Synthesis and biological investigations of alkylating agent-RGD peptide conjugates (Süli-Vargha, Giavazzi).

2003

1. NQO1-Targeted prodrugs for cancer therapy: In silico screening of virtual libraries and identification of novel agents (Jaffar, Bibby, Stratford).
2. Preparation, characterization and in vivo testing for antitumor activity of a candidate pyrrolo-tetrazinone (Cirrincione, Fichtner).

2005

1. In vitro and in vivo activity and mechanism of action of multi and duplex drugs (Fichtner, Schwendener, Schott, Peters).

2006

1. Synthesis and evaluation of selective inhibitors of the E3 ubiquitin ligase BCA2 for potential treatment of invasive breast cancer (Westwell, Burger).

2009

1. Synthesis and biological evaluation of inhibitors of human lactate dehydrogenase 5 as cancer starvation agents (Minutolo, Peters).

molecular combinations of 5-FU and BCNU (B.3839) (1986),[2] CNC-L-alanine-dihydrotestosteron-17-ester (E91) (1987), amino acid carriers of chloroethyl-nitrosoureas (E10, 79, 94, 126, 127) (1987), molecular combinations of 5-FU and nitrosoureas (B.3995 and B.3996) (1988), and imidazoacridinone C-1311 (1997). Research on these drugs was often supported by mini projects which were funded out of the group's income (until 2000 the annual contribution of the EORTC with occasionally grants from the NCI), and intended to support drug development with feeder money (Table 3). Several compounds developed within the framework of the

SPG and often in collaboration with PAMM went into clinical development: Flavone acetic acid,[3] EO9 (Oostveen, Pinedo), AQ4N (Laurence Patterson), temozolomide (Stevens), imidazoacridinone C-1311 (Konopa), nucleoside prodrugs, Patrin2, Phortress[4] (Stevens, Bradshaw, Westwell); 2-(4-amino-3-methylphenyl)-5-fluorobenzothiazole L-lysylamide dihydrochloride.[5] Several of these drugs will be discussed in more detail in the following.

2.1. Development of temozolomide

Temozolomide was synthesized as a DNA interacting agent by Prof. Malcolm Stevens (then at Aston University, Birmingham; from 1992 at the University of Nottingham, and SPG Chair from 1987 to 1990) and initially tested within the facilities of the SPG. Although *in vitro* activity was minor, it was the belief of the group that this would not be important because it was designed to be activated *in vivo*.[6] These studies also led to the finding that temozolomide could pass the blood–brain barrier efficiently and proved to be active against animal brain tumor models. Pivotal studies by the late Ed Newlands showed a good activity against glioblastoma.[7,8] Randomized studies from the EORTC Brain Tumor Group clearly showed an advantage of temozolomide combined with radiation compared with radiation alone and formed the basis for its registration.[9] This study also demonstrated the important role of methylation of the MGMT gene promoter.[10]

2.2. Prodrugs, mutidrugs and drug carriers

Many drugs in development are administered in a suboptimal way: they are either insoluble, have a short half-life, have a poor uptake, or are extensively catabolized to toxic breakdown products. Even many commonly used, successful drugs are not always given in an optimal way. Therefore several SPG members, often in collaboration with other SPG or PAMM members or a pharmaceutical company, investigated several ways to optimize administration. When drugs are combined, either as two active drugs, modulating agents, or co-medication, these effects are either diminished or even increased. In order to improve delivery or bypass unwanted side-effects several attempts have been pursued. Drug delivery has been improved by administration of the drugs in liposomes, nanoparticles, antibody-directed enzyme prodrug therapy (ADEPT), or more recently by gene-directed enzyme prodrug therapy (GDEPT).

With ADEPT an enzyme is coupled to an antibody which ideally will specifically recognize a tumor cell. This is delivered together with an inactive prodrug, systemically, which is specifically activated by this enzyme at the tumor site to become a toxic drug. With GDEPT a gene is delivered with the information to produce this enzyme in the tumor, so that the inactive prodrug can be converted into the toxic drug. Several investigators within the SPG and PAMM worked on this concept with nitroreductase as the best described example. Nitroreductase from *E. coli* can activate the prodrug CB1954 to a bifunctional DNA cross-linking drug enzyme, a concept which was developed at the ICR.[11,12]

Other prodrugs were developed to improve delivery and uptake by cancer cells, since a frequently observed resistance mechanism for nucleoside analogs is decreased uptake. Hence prodrugs of cytarabine and gemcitabine were developed by attachment of a fatty acid tail (elaidic acid) to the sugar moiety; the ara-C-prodrug elacytarabine had impressive activity against solid tumor xenografts, while ara-C does not.[13] The gemcitabine prodrug CP-4126 (now developed as CO1.01) had a similar profile as gemcitabine but could also be given orally.[14] Both drugs bypassed resistance due to nucleoside transport deficiency, were developed in collaboration between the EORTC New Drug Development Office and several SPG laboratories, and are in Phase II and III clinical trials. Other approaches included multidrugs, two drugs linked by a lipid which would enhance drug delivery, with the advantage of delivering two cytotoxic drugs to the tumor simultaneously. Nanoparticles have also been investigated by the SPG and the DDC.

2.3. The NCI compounds initiative

In 1993 an important collaboration was established between the SPG and PAMM with the NCI and CRC (Cancer Research Campaign, now Cancer Research UK), the so-called NCI compounds initiative. These joint forces aimed to speed up the development process of NCEs in Europe with emphasis on compounds synthesized in Europe, especially compounds synthesized by groups unfamiliar with the drug development process. The intention of this initiative was to join forces on the various aspects of drug development: initial activity screening, formulation, mechanism of action, toxicology, and preclinical pharmacokinetics, with an emphasis on novel drug entities. The procedure involved an initial screen of compounds tested by the NCI in the 60-cell line panel, the hollow-fiber assay and/or xenografts. Based on the novelty of the structural formula, the selective activity in the 60-cell lines panel, the uniqueness in the COMPARE analysis, the *in vivo* activity and the chemical properties, a decision was made whether the NCE was worthwhile to develop and by whom. Because of the insolubility of many compounds, formulation became a major effort to ensure reliable administration.[15] This also required a different approach for drug analysis, but due to the expertise of the many collaborating centers, this was usually solved, and this expertise also enabled evaluation of many classes of drugs including drugs targeted against cytotoxic targets such as novel nitrogen mustards, antimetabolites, DNA

intercalators, tubulin-directed drugs, and more recently, non-toxic drugs targeting signal transduction, tumor physiology, and angiogenesis. This effort led to many fruitful collaborations often facilitated by the laboratories themselves. Furthermore, accumulating insight in drug development of specific classes led to omission of certain groups, such as tubulin-directed drugs. The NCI always actively participated in this joint effort by attending the NCI compounds meeting, providing the drug information, the initial drug screening, active discussions and financial support. The CRC provided support for drug screening.

3. The EORTC Clonogenic Assay Study Group (CASG)

The CASG was formed in 1981 as the CASC in order to coordinate European activities on the use of the clonogenic assay as a tool to select which drug would be the most suitable for a certain patient. This research was initiated following the seminal publication of Salmon-Hamburger [16] on the use of the clonogenic assay to select drugs for individual patients. Both in the United States and in Europe this idea was received enthusiastically, because it represented one of the first attempts to allow personalized treatment. The rationale behind this principle was novel at that time and similar to current stem cell theories. It was reasoned that not all tumor cells would contribute to the development of a tumor, but that only a few "very malignant" cells would lead to tumor cell formation. In the current terminology these cells would be called cancer stem cells. Hence, selecting the drug which would kill this specific cell would most likely be the most active drug against the tumor from the patients. Therefore, many laboratories collected primary tumors from patients during surgery, isolated tumor cells and plated these cells on agar in order to form colonies. The plating efficiency of the clonogenic assay was usually less than 1 out of 10,000 or 100,000 or even less, similar to the low abundance of stem cells. A number of laboratories were very successful, leading to spin-off companies who still use a modernized form of the clonogenic assay to test for drug activity. However, despite the simplicity of the idea, execution of the clonogenic assay encountered many problems. The CASG took this challenge and established a European forum in order to validate the clonogenic assay across European laboratories for a number of cell lines but always in comparison with the colon carcinoma cell line WiDr. This type of validation was a hallmark of the group. [17,18] The clonogenic assay was routinely performed on agar or methyl cellulose, while the used media consisted of many unknown additions, such as bacterial extracts, various types of serum, growth factors extracted from tumor fluids, such as ascites or pleural effusions. Although this type of research did not yield reproducible data when compared between laboratories, within several laboratories in which the methodology was validated very impressive correlations between the inhibition of colony formation and therapeutic efficacy were found. Furthermore, characterization of the various unknown proteins considered to be essential for colony formation led to the identification of many currently well-known growth factors, such as Vascular Endothelial Growth Factor, Insulin Growth Factor, Platelet-Derived Growth Factor, Epidermal Growth Factor and others. [19]

Because of the above-mentioned challenges, the CASG decided to extend its focus and changed its name. In 1991 it was called In vitro Study Group, but in 1993 it changed its name to the PTMG to use other model systems as well, such as two-dimensional monolayer cultures and suspension cell lines in comparison with other three-dimensional culture systems such as spheroids, or multilayer cultures, and other types of assays such as the SRB, MTT and ATP assay. [20,21] The easy availability of different cell lines in the participating laboratories enabled studies in many cell lines, similar to the NCI 60-cell line panel with the WiDr cell line as the golden standard. Screening efforts were naturally accompanied by mechanistic studies, completely in line with the aims of PAMM, leading to the merging with PAMM. Hence, the last joint PTMG paper was a PAMM paper as well. [22]

4. The EORTC Pharmacokinetics and Metabolism Group (PAM)

The first meetings of PAM focused on development of suitable assays for the many new drugs which were introduced in the 1970's and 1980's. The relatively new technique of HPLC offered an opportunity for reliable, sensitive, and reproducible drug measurements and performance of pharmacokinetic studies in phase I and II studies. Also other techniques were introduced, such as Radio-Immuno-Assays, and Flameless Atomic Absorption Spectroscopy which was essential to understand the pharmacology and molecular mechanisms of not only cisplatin but also its novel analogs such carboplatin and oxaliplatin, and to understand why other analogs such TNO-6 were too toxic. It is not surprising that several early PAM members (H.M. Pinedo, J.F. Smyth) were intensively involved in the ECTG, and many ECTG members remained PAM (later PAMM) members. This interaction between clinicians and scientists was unique in the 1980's and formed a firm basis for inclusion of translational research in these clinical studies. These studies were often a spin-off of mechanistic studies towards the metabolism of these novel anticancer drugs.

4.1. *Pharmacokinetics-guided dose escalation and drug administration*

Development of anticancer drugs from animals to patients is a tedious process, in which the limiting factor for any drug is its tolerability in patients. Therefore the escalation steps, usually following a modified Fibonacci scheme, are very conservative with careful monitoring of all patients (see a monograph on these aspects with many PAM/PAMM contributions [23]). This means that many patients are initially being dosed at very low levels, and that it takes many steps to reach the maximum tolerated dose (MTD). Therefore the PAM initially drafted a number of guidelines in order to streamline the drug dose escalation within EORTC phase I trials. Using mouse models, an algorithm was developed which based the first dosing step on the MTD in mice (first dose 1/10 of the LD10 in mice), while the initial dose escalation was based on the mouse pharmacokinetics (PK), naturally accompanied by careful monitoring of human PK. [24,25] This required a stringent quality assurance of assays, always a major aspect of PAMM activities. The initial studies faced a number of challenges, [26] but various drugs were entered into the clinic and were developed according to this algorithm which still forms the basis for drug escalation in Phase I studies, albeit with several modifications.

Examples of these drugs include several alkylating agents, anthracyclines, platinum analogs and antimetabolites, [27] although it was assumed that the concept would be less accurate for antimetabolites. However, despite a short plasma half-life, antimetabolites often have a much longer tissue half-life due to extensive metabolism leading to trapping in the tissues.

4.2. *Development of novel platinum analogs*

The excitement following the introduction of cisplatin in the 1970's which led to cure of several previously incurable diseases (e.g. testicular cancer) was dampened by the serious side effects. Hence efforts of several PAM and SPG laboratories were directed to develop novel platinum analogs in close collaboration with Johnson Matthey and several pharmaceutical companies, such as Bristol-Myers, while several SPG chemists were involved as well. [28] This led to the development of a series of "JM-platinums" and other series, such as the TNO compounds. [29] The ICR played a seminal role combining chemistry, testing in model systems, and clinical studies. This was the start of the career of the late Lloyd Kelland, who was responsible for testing many of these analogs. [30] There was a fruitful cross-fertilization leading to several other series of compounds such as the TNO series but also other types of platinum complexes. Unfortunately, many of these compounds were either inactive or were associated with too many side effects such as TNO-6, co-developed by TNO in Rijswijk and the VU University Medical Center in Amsterdam. A successful example of these joint efforts is carboplatin, while satraplatin (JM-216) is still a potential candidate for oral application of a platinum analog.

4.3. *Personalized treatment with carboplatin*

In contrast to cisplatin, which carries a major risk of nephrotoxicity, the development of carboplatin was hampered by the development of myelotoxicity. However, already at an early stage it was recognized that this toxicity was exposure dependent. The increasing insight in the association of PK–PD relationships was used to define the association between carboplatin pharmacokinetics, clearance, and thrombocytopenia in a clinical study coordinated by Hilary Calvert (then at the Institute of Cancer research, Sutton, Surrey) together with Herbie Newell, PAMM chair from 1994 to 1997. This led to a simple formula, the so-called Calvert formula, still widely used to dose carboplatin, [31] in which the clearance is used to dose carboplatin in order to get the target area-under-the curve (AUC) and thereby prevent excessive toxicity and maximize the efficacy. This can be considered as one of the earlier forms of personalized treatment. Because of the inaccuracy of the assays for clearance, the change in methodology, and extensive use in combinations a number of adaptations have been made. Furthermore, the formula has been adapted for use in various groups of patients including young patients. [32] Population pharmacokinetic analysis of carboplatin helped to define the differences between these populations and was performed to optimize the algorithm.

5. The Pharmacology and Molecular Mechanism Group (PAMM)

The change in medicinal chemistry, analytical techniques, imaging techniques, molecular biology, and cell biology in the 1980's and early 1990's led to a shift in the way drugs were developed, and most importantly, the classes of drugs were changing as well. The challenge of the last decade included the question of how to evaluate novel classes of drugs such as several anti-angiogenic drugs and tyrosine kinase inhibitors. Hence the emphasis of the PAM meetings changed from classical pharmacokinetics to many additional aspects of pharmacology (including pharmacodynamics, later pharmacogenetics, but also cellular pharmacology and molecular pharmacology). Therefore it was decided to change the name to Pharmacology and Molecular Mechanisms (PAMM). PAMM became involved in drugs targeting signaling, reversal of multidrug resistance, novel DNA intercalators, drugs from natural resources, drugs targeting growth factors, prodrug activation systems, drugs targeting the protein degradation and recycling (proteasome, heat-shock proteins, aminopeptidase, farnesylation), [33] p53

and other apoptosis-directed drugs, several types of prodrugs and formulations,[34] and chemoprevention. The intensive interaction with the SPG and PTMG members enabled the use of many model systems to investigate drug action and develop potential biomarkers alongside the clinical and preclinical studies. Some examples of these drug classes are discussed in more detail below.

5.1. *Development of EO9 (EOquin, apaziquone)*

Initially, EO9 was jointly developed by the University of Amsterdam and the VU University, but it rapidly became a joint effort of PAMM, SPG and the EORTC New Drug Development Office in which a number of their laboratories became involved.[35] It was part of a series of indoloquinone derivatives of mitomycin. Its favorable activity in a number of preclinical *in vitro* and *in vivo* models led to its selection for further development. EO9 is a prodrug belonging to the class of bioreductive agents and needs to be activated by an oxidoreductase in order to generate cytotoxic metabolites[36]; the activity of the drug is related to the activation of DT-diaphorase (NQO1) in normoxic tumors, but it is preferentially activated in hypoxic tumors.[37,38] Unfortunately, the Phase I and II studies were negative because of a very poor pharmacokinetic profile with a very short half-life preventing the drug being taken up by tumor tissues.[39,40] Because of the poor drug delivery profile, further development turned this into an advantage, and the drug was tested in the treatment of superficial bladder cancer.[41] The longer local exposure of these tumors not only permitted drug penetration, but the hypoxic conditions were in favor of this drug as well; poor plasma pharmacokinetics would prevent any systemic drug accumulation and toxicity. In several Phase II studies complete responses in up to 2/3 of the patients were achieved.[42] Recurrences are infrequent, and the drug is currently further developed for local treatment of superficial bladder cancer. The development of this drug demonstrates that proper development which takes all aspects into account can change poor PK profiles into an advantage.

5.2. *Development of antifolates*

Antifolates are one of the oldest classes of anticancer drugs, with methotrexate as the prototype. However, the efficacy of classical antifolates is limited by their uptake (mediated by either the folate receptor [FR], reduced folate carrier [RFC], and the recently characterized proton-coupled folate transporter], efflux by RFC or ABC pumps, metabolism to a polyglutamate form by folylpolyglutamate synthetase (FPGS), and target modification (either dihydrofolate reductase [DHFR] or thymidylate synthase [TS]). In order to improve the efficacy of this class of drugs, the ICR and other groups developed a number of folate analogs, but instead of targeting DHFR, these drugs were designed to inhibit TS which is also the target for 5-fluorouracil (5-FU). The first prototype, CB3717, showed excellent TS inhibition and was a good substrate for FR and RFC, although uptake was predominantly dependent on the RFC.[43] Unfortunately, the drug showed serious nephrotoxicity,[44] but modification of the molecule led to the development of Tomudex™ (raltitrexed), which is an excellent substrate for RFC and FPGS, while the polyglutamates are very effective TS inhibitors. Tomudex's mechanism of action studies were a joint PAMM effort, predominantly between Amsterdam and Sutton,[45] while clinical studies were performed by the ECTG[46] and later by other groups such as the EORTC Gastrointestinal Tract Cancer and Lung Cancer Groups.[47] This led to the registration of Tomudex for the treatment of colon cancer. Current efforts focus on drugs which either do not need polyglutamylation (Plevitrexed, ZD 9331) or are dependent on the FR (BGC945).[48] The latest compound has been shown to be selectively active in tumors with a high FR expression and is currently evaluated in ovarian cancer which has high FR expression.

5.3. *Imidazoacridinones*

Another nice example in which all groups (SPG, PTMG and PAMM) worked intensively together was the development of C-1311 (Symadex™). C-1311 belongs to the group of imidazoacridinones which were rationally designed by the Gdansk group of Prof. Konopa on the basis of structure–activity relationship studies on mitoxantrone.[49] The most promising analogue, C-1311, which contains the hydroxyl group in position 8 of the imidazoacridinone core, is currently in phase II clinical trial. C-1311 is rapidly transported to tumor cells, accumulates in the nucleus, and has shown potent activity against experimental models of murine and human colorectal cancer *in vitro* and in animal studies carried out by the SPG. The drug, which undergoes enzymatic oxidative activation, has been shown to intercalate into DNA and trap topoisomerase II cleavable complexes[50] but is extruded by the multidrug resistance protein ABCG2.[51] These mechanistic studies were performed by various PAMM, SPG and PTMG members in which the late Angelika Burger was instrumental. It was shown that C-1311 treatment resulted in cell death but failed to induce apoptosis, but cell death was possibly induced in a process resembling abortive mitosis or mitotic catastrophe.

5.4. *Trabectedin*

Trabectedin (Yondelis®, also known as ecteinascidin 743 or ET-743) is a small molecule which occurs naturally in the Caribbean tunicate *Ecteinascidia turbinate*. The initial development of trabectedin was performed using the natural product. PharmaMar developed the chemical

synthesis of the compound used in phase II studies. Trabectedin showed striking antitumor activity in preclinical rodent models. [52] Various PAMM participants contributed significantly to this development predominantly focusing on its mechanism of action. The compound binds in the minor groove of DNA and shows a unique mode of action. Studies performed by laboratories taking part in PAMM (D'Incalci, Milan; Larsen, Paris) have identified a unique pattern of response of the drug depending on the function of different DNA repair pathways. The most sensitive cancer cells are those with an efficient nucleotide excision repair activity and a dysfunction of homologous recombination. These findings have been confirmed in the clinic in sarcoma patients treated with trabectedin in trials conducted by the EORTC Soft Tissue and Bone Sarcoma Group. In addition, the laboratory headed by D'Incalci, Milan, has demonstrated that trabectedin is a modulator of transcription regulation of some cancer-related genes. These peculiar promoter- and gene-dependent transcription effects explain the very high activity of trabectedin against some sarcomas whose pathogenesis is related to the deregulation of a specific transcription factor (e.g. myxoid liposarcoma). In addition, the same researchers (D'Incalci, Giavazzi, Larsen) have demonstrated that trabectedin is very effective against ovarian cancer xenografts resistant to other anticancer drugs, such as cisplatin, and has additive to synergistic effects in combination with cisplatin. [53,54] These data have provided the rationale for phase II studies in ovarian cancer that have confirmed results obtained in the laboratory. Beinen's laboratory in Amsterdam has developed a highly sensitive and specific HPLC-MS method for the determination of trabectedin in biological fluids. This method was successfully applied to characterize the clinical pharmacokinetics of trabectedin during the early phase of its development. [55]

In 2007 the European Medicine Evaluation Agency (EMA) approved trabectedin for the treatment of soft-tissue sarcomas and in 2009 for the treatment of ovarian cancer. Myelosuppression is the principal dose-limiting side-effect of the drug, but hepatotoxicity has been frequently observed in the clinic, as well. Several PAMM groups (Gescher, Leicester; D'Incalci, Milan) collaborated with PharmaMar to biochemically and pathologically characterize this toxicity in the rat, after which it was hypothesized that the glucocorticoid dexamethasone can influence trabectedin hepatoxicity. [56] High-dose dexamethasone administered before trabectedin dramatically abrogated trabectedin hepatotoxity in rodents without confounding its antitumor activity, a finding which was translated to the clinic with the same result, [57] leading to standard application of dexamethasone in combination with trabectedin.

5.5. *The BIOMED program*

In line with the traditions of the original PAM group, Jan Schellens initiated a joint European program which was funded by the EU in the BIOMED 2 call. The aim of the grant was to establish a European consortium which would finally perform a population pharmacokinetic program on several widely used anticancer drugs in order to optimize the treatment with these drugs. This program involved a number of important steps, including the characterization and motivation of several PAMM laboratories, several large clinical centers which would be willing and able to recruit sufficient patients for this program, cross-validation of the laboratories, and implementation of the data. The persistence of the coordinator Jan Schellens and the various PAMM collaborators (Karlsson, Uppsala; Boddy & Newell, Newcastle; Chatelut, Toulouse; Van der Vijgh & Vermorken, Amsterdam; Bruno), led to a successful program despite the fact that not all patients could be included in the program, especially because of various competing Phase I and Phase II studies. This collaborative program demonstrated the feasibility of sampling in various centers, cross-validation of analytical techniques, and an association of length of paclitaxel exposure with outcome and carboplatin AUC with toxicity. [58,59] Drug monitoring and population pharmacokinetics are recurring items at PAMM meetings. [60]

5.6. *Inhibitors of signaling pathways*

Early on PAMM recognized the importance of novel antisignaling drugs, in which one of the early PAM chairs, Paul Workman (now Director of the Cancer Therapeutics Unit and Deputy Chief Executive of The Institute of Cancer Research) played a key role. [61,62] Joint AACR, BACR, and PAM meetings were organized for this purpose. [63,64] The early focus was on protein kinase C signaling and related cyclic nucleotides and on how to combine them with cytotoxic drugs. [65] The importance of tyrosine kinases in activation of growth factor receptors and angiogenesis was recognized, especially because of the expertise in the SPG group in animal models for angiogenesis. Subsequently several prominent PAMM members were involved in the introduction of several new tyrosine kinase inhibitors into the clinic including several predecessors of sunitinib such as SU5416. The negative data with SU5416, especially toxicity, were invaluable for successful development of newer analogs, such as sunitinib.

6. Multidrug resistance

PAM, SPG, PTMG and later PAMM have always been heavily involved in resistance, including multidrug resistance. This is not surprising since the M of PAM

stands for metabolism and the two MMs of PAMM for molecular mechanisms, which form the basis for any resistance genotype and phenotype. PAMM meetings repeatedly discussed resistance to common drugs like the classes of antimetabolites, platinum, anthracyclines, taxanes, imdazoacridones, and recently that of tyrosine kinase inhibitors. The role of multidrug resistance in these classes of drugs has been investigated intensively in many collaborative studies of PAMM members.[66] Unfortunately, the investigations on P-glycoprotein (PgP) reverters of drug resistance of tumors were not very successful in the clinic, since the compounds did not improve antitumor activity of explored drugs such as anthracyclines. However, this insight into PgP led to a better understanding of the mechanism of penetration of drugs into the brain and on the role of these pumps in drug absorption from the gut. The investigation of PgP inhibitors in order to facilitate oral administration of drugs which are PgP substrates, holds more promise than the resistance-reverter studies. More recently the role of several MRPs (ABCB1, etc.) and BCRP (ABCG2) in resistance was widely explored, and it was concluded in several investigations that these phenomena are more likely to be related to clinical resistance.

7. The new area of pharmacogenetics and pharmacogenomics

The PAM and PAMM have always been very much involved in investigations on tumor metabolism of anticancer drugs and the role of metabolites in the antitumor activity and toxicity of both classical anticancer drugs but also the new generation of antisignaling drugs. PAM was one of the first groups to establish PK–PD relationships which were rapidly reported to be associated with genetic defects of polymorphisms. Well investigated examples are the role of impaired 5-FU degradation by dihydropyrimidine dehydrogenase in sometimes lethal 5-FU toxicity; especially French[67] and Dutch[68] groups were active in this field. More recently this field has been extended to the investigation of another antimetabolite, gemcitabine. Deficiency of its degradation pathway (cytidine deaminase) was found to be associated with increased side-effects but also with a better efficacy.[69,70] A collaboration between Italian, French, Dutch and English groups to validate the detection is ongoing. These studies were initiated by measurement of enzyme activities and aberrant pharmacokinetics, but rapidly it appeared that these enzyme deficiencies were associated either with the absence of gene expression or with specific genetic polymorphisms. Similar associations have now been described, and several times also implemented, for other drugs and enzymes, such as CYP450 enzymes (irinotecan), various transporters (e.g. ABCG2), glucuronidation, esterases, etc.

8. Conclusions and future perspectives

PAMM has a tremendous expertise in the study of drug metabolism, pharmacokinetics and the role in drug action. It performed translational studies from the beginning of its existence and has contributed to the standardization of protocols for drug testing and screening, phase I study methodology and early protocols for personalized treatment. The hallmark of PAMM, interaction between clinical researchers and laboratory scientists, has been essential for these achievements. Currently this interaction is increasingly considered to be essential for successful drug development. In the near future PAMM will continue research on drug screening and drug development in collaboration with other groups including NCI, CRUK, GPCO, SENDO, and CESAR. PAMM will continue to explore how implementation of proper pharmacokinetics can enhance drug development (see EO9) but will also explore how novel approaches with pharmacogenetic and pharmacogenomic profiling will help to personalize treatment. Interaction with the EORTC Pathobiology and Imaging Groups has intensified and will be essential to achieve these goals. In the last few years the interaction with the EORTC Disease Oriented Groups has replaced that with the ECTG and NDDP and several collaborative projects are now being implemented.

9. Acknowledgements

The authors wish to thank the previous PAM/PAMM/SPG/PTMG chairs and secretaries and the current PAMM committee members for their constructive comments on the draft of this article, especially on early important events in the history of the various groups.

10. Conflict of interest statement

E. Chatelut and N. Zaffaroni declare no conflicts of interest. G.J. Peters consulted for, and received research funds from, Clavis Pharma, consulted for Spectrum, and received research funds from Taiho. A.K. Larsen received research funds from PharmaMar.

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