

European Association of NeuroOncology Magazine

Neurology · Neurosurgery · Medical Oncology · Radiotherapy · Paediatric Neuro-oncology · Neuropathology · Neuroradiology · Neuroimaging · Nursing · Patient Issues

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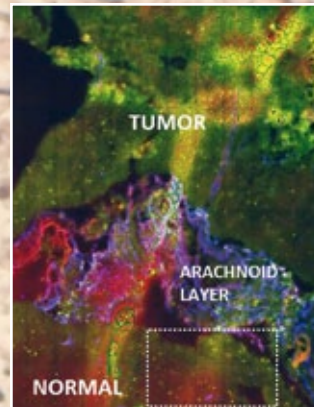
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Editorial

Dear EANO members, dear colleagues,

several events have occurred since my editorial in the previous issue of *EANO Magazine*.

At the last EANO board meeting in Vienna on December 4, 2013, we agreed to support 2 young researchers with a grant for performing a Cochrane review on “Fatigue” and “Particle vs photon radiotherapy in chordomas”, respectively.

The 4th Quadrennial meeting of the World Federation of Neuro-Oncology (WFNO) in San Francisco (November 21–24, 2013) was very successful and the contribution of Europe in terms of invited speakers/chairs or original presentations was high both numerically and scientifically. The 5th WFNO meeting will be organised by EANO in Switzerland (Zurich or Geneva) in the spring of 2017.

The EANO guidelines on malignant gliomas have been finalized and accepted for publication in *Lancet Oncology*, while the Task Force on Guidelines on PCNSL is still working and the document will be hopefully ready within the next few months. Meantime, a Task Force on the Management of Brain Metastases is being developed in order to produce guidelines by the end of this year.

The organisation of EANO 2014 in Turin (October 9–12) has reached an advanced stage. Online abstract submission will be open until March 21, and the programme of the educational day, plenary and meet-the-expert sessions is ready and available at www.eano.eu. The EANO 2016 meeting will be organised by the German Group of Neuro-Oncology in Heidelberg/Mannheim in September or October 2016.

In Turin, I will resign from my position as EANO president and Michael Weller from Zurich has been appointed as president-elect. Moreover, at the end of March, we will launch on the website a call for the election of new board and scientific committee members to be finalised in the general assembly during the EANO meeting in Turin.

Last, over the past months, downloads and subscriptions for *EANO Magazine* have further increased. Overall, we would be very happy to receive either contributions or suggestions from EANO members in order to improve the quality of information and to try to meet as much as possible the needs of European people involved in neuro-oncological activities.

Best regards,

Riccardo Soffietti
EANO President 2012–2014

Introduction

In this edition of *EANO Magazine*, the “Multidisciplinary oncology guidelines: report of the ECCO Forum Multidisciplinary Clinical Guidelines” are published and present the results of meetings by representatives of several cancer organisations. The aim was to define quality criteria to develop and identify high-quality multidisciplinary guidelines. It was decided that ECCO would not develop guidelines by themselves, but act as a “switchboard” to avoid redundancies, to review and endorse multidisciplinary guidelines fulfilling the quality criteria, and to disseminate endorsed guidelines. Last but not least ECCO will represent the voice of oncology guidelines at the EU policy level.

Guidelines are statements determining a course of action and are increasingly used in medical and oncologic procedures. The techniques to obtain guidelines are getting more sophisticated, and in addition to levels of evidence and good clinical practice sound quality criteria need to be available. Guidelines need to be adaptive to new developments and they must be feasible as well as acceptable to European health systems and stake holders, to be available and useful for individual patients.

The meeting of different representatives of cancer organisations has shown several important aspects: (1) different and diverse needs for different fields with regard to guidelines and (2) the different methodologies used and the already existing body of evidence for guidelines, which vary between the different cancer organisations.

Two other aspects are important and need to be developed further: (1) the multidisciplinary approach and also (2) the involvement of patient representatives, which is an important and long necessary trend which has been implemented in this document.

EANO is part of this document and fully supports this development. EANO’s open access journal publishes this document, which will be openly accessible or can be linked into any of the ECCO organisations.

Wolfgang Grisold, MD
Riccardo Soffietti, MD

Quality Criteria for Multidisciplinary Oncology Guidelines – the ECCO Multidisciplinary Clinical Guidelines Forum

Dirk Schrijvers¹, Cornelis van den Velde¹, Marco Bregni², Augusto Caraceni³, Fatima Cardoso⁴, Sema Erdem⁵, Hans Gelderblom⁶, Wolfgang Grisold⁷, Per Gustaf Hartvig Honore⁸, Bernd Krause⁹, Lisa Licitra¹⁰, Robert Mansel¹¹, Anita Margulies¹², Keith Parsons¹³, Graeme Poston¹⁴, Vincenzo Valentini¹⁵, Paolo Zola¹⁶

Abstract: A multidisciplinary oncology guideline is defined as a guideline developed by representatives of (cancer) organisations and deals with cancer prevention, screening, diagnosis, treatment, and care as well as quality and safety of the involved stakeholders. ECCO organized a

forum with its members and other oncology organisations to define quality criteria for the development groups, partners, formats, and quality measures to be recognized by ECCO as high-quality multidisciplinary guidelines. **Eur Assoc NeuroOncol Mag 2014; 4 (1): 6–8.**

Key words: ECCO, multidisciplinary oncology guidelines, development group, partners, quality control

■ Introduction

Guidelines are tools to help oncologists in clinical practice ensure optimal patient care. Oncology treatment and care have become multidisciplinary and guideline development groups should reflect this trend to provide up-to-date multidisciplinary cancer care.

Many European organisations are producing guidelines in the field of oncology. In a survey on guideline development including 30 European organisations involved in cancer care, 62 % of the 21 responding organisations were developing guidelines, mainly focusing on cancer diagnosis and treatment/management to ensure adequate clinical care. Many of them worked with a multidisciplinary team and 74 % involved patient representatives. For most development groups there was no formal training but different forms of quality control were applied to ensure validity of the guidelines. Guideline development puts a heavy financial burden on the developing society with a median cost per guideline of € 25,000–50,000 [1].

Health care provision differs among European countries and cancer care can be different among European countries and regions. This has an influence on cancer outcome [2] and

highlights the importance of guidelines. European guidelines based on internationally accepted criteria can be used as bases for local health care organisations to develop national or regional guidelines to improve cancer care and treatment.

The European CanCer Organisation (ECCO) organised a “Multidisciplinary Clinical Guidelines Forum Working Group” meeting to improve European multidisciplinary oncology guideline development and stimulate cooperation among development groups. A multidisciplinary oncology guideline was defined as a guideline developed by representatives of (cancer) organisations and dealing with cancer prevention, screening, diagnosis, treatment, and care as well as quality and safety of the involved stakeholders. The aim of the meeting was to define quality criteria for the development groups, partners, formats, and quality measures so that they could be endorsed by ECCO as high-quality multidisciplinary guidelines. Also, the role of ECCO in the guideline development and implementation process was discussed.

■ Methodology and Participants

ECCO invited different ECCO organisations and other organisations involved in cancer care and participating in the previous guideline project [1] to attend the forum. Together with the invitation, a questionnaire on procedures for (multidisciplinary) guideline development, templates and formats, and quality instruments of guidelines (Table 1) was sent to be returned before the forum meeting to serve as a discussion tool. The “forum” took place on November 27, 2012.

The meeting was structured to encourage interaction and discussion. After an introduction, different organisations made presentations on their guideline development process, followed by a group discussion on different topics, including a standard operating procedure/checklist with quality indicators for the development of multidisciplinary guidelines and the strengths/added value of an endorsement process by ECCO.

A set of conclusions was proposed and a meeting report of the forum was written and distributed among the different organisations for remarks. A final document [3] for approval by the

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¹On behalf of the European CanCer Organisation (ECCO); ²on behalf of the European Group for Blood and Marrow Transplantation (EBMT); ³on behalf of the European Association of Palliative Care (EAPC); ⁴on behalf of the European School of Oncology (ESO); ⁵on behalf of the European CanCer Organisation Patient Advisory Committee (ECCO PAC); ⁶on behalf of the European Organisation for Research and Treatment of Cancer (EORTC); ⁷on behalf of the European Association of Neuro-Oncology (EANO); ⁸on behalf of the European Society of Oncology Pharmacy (ESOP); ⁹on behalf of the European Association of Nuclear Medicine (EANM); ¹⁰on behalf of the Organisation of European Cancer Institutes (OEI); ¹¹on behalf of the European Society of Breast Cancer Specialists (EUSOMA); ¹²on behalf of the European Oncology Nursing Society (EONS); ¹³on behalf of the European Association of Urology (EAU); ¹⁴on behalf of the European Society of Surgical Oncology (ESSO); ¹⁵on behalf of the European Society for Radiotherapy and Oncology (ESTRO); ¹⁶on behalf of the European Society of Gynaecological Oncology (ESGO)

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Table 1. Organisations participating in the Multidisciplinary Clinical Guidelines Forum

Item/Question	Yes	No
Present during the forum	EANM, EANO, EAPC, EAU, EBMT, ECCO, ECCO PAC, EONS, EORTC, ESGO, ESMO, ESSO, EUSOMA, OEI, EPAAC	ESO, ESOP, ESTRO, UICC
1. Is your society involved in guideline development?	ESSO, EANM, ESO, EANO, EUSOMA, EAU, EAPC, EONS, ESGO, ESMO, ESTRO, ESOP, EORTC	OEI, EBMT
2. Do you have a specific procedure for guideline development?	EAU, EANM, ESMO, EANO, ESOP, ESGO, EUSOMA, ESO, ESTRO (in development), ESSO, EORTC, EAPC	OEI, EBMT
3. Do you use a procedure for guideline development based on national/international criteria (eg, NICE, WHO, others)?	EAU (partial), ESMO, EANO (EFNS), ESOP (German Oncology Society), EUSOMA, ESSO, EAPC, ESGO	ESO, EORTC, OEI, EANM, ESTRO, EBMT
4. Do you produce multidisciplinary guidelines?	ESSO, EANM, EAU, EANO, EONS, EAPC, ESMO, ESTRO, ESOP, EORTC, ESO, EUSOMA	OEI, ESGO, EBMT
5. Do you produce multidisciplinary guidelines with the involvement of experts delegated by other ECCO member societies?	EAU (not ECCO), ESMO, ESTRO, ESOP, ESO, EUSOMA, ESSO, EANM	EANO, EAPC, OEI, ESGO, EBMT, EORTC
6. Do you have a system to contact ECCO member societies for participation in guideline development?	ESMO, ESSO, EANM, ESTRO	EAU, OEI, EONS, ESGO, ESO, EBMT, EANO, EORTC, EAPC, ESOP, EUSOMA
7. Do you have a specific template to publish the guideline?	ESMO, EANM, EAU, OEI, EUSOMA, EONS, ESGO, ESOP, ESTRO, ESSO, EANO (EFNS)	EONS, ESO, EAPC, EBMT, EORTC
8. Do you have a full-text format template of the guideline?	ESMO, ESO, ESOP, ESSO, EANO, OEI, ESTRO, EUSOMA	EAU, EANM, EONS, EAPC, ESGO, EBMT, EORTC
9. Do you have a flow diagram format of the guideline?	ESMO, ESGO	EAU, EANM, EONS, EAPC, ESOP, OEI, ESO, ESSO, ESTRO, EANO, EBMT, EORTC, EUSOMA
10. Do you have a patient format of the guideline?	EAPC, EAU (under development), ESMO, ESSO, OEI	EONS, ESGO, ESOP, ESTRO, ESO, EBMT, EANM, EANO, EORTC, EUSOMA
11. Do you have other formats of the guideline?	EAU, ESO, ESSO, EAPC, ESGO	ESMO, EANM, EANO, ESTRO, EBMT, EORTC, EUSOMA
12. Do you have a quality evaluation instrument of the guideline?	ESMO, ESTRO, ESOP, ESSO, OEI, ESGO	EAU (not routine), EONS, EAPC, ESO, EBMT, EANM, EANO, EORTC, EUSOMA
13. Do you use the AGREE instrument for quality evaluation of the guideline?	ESGO, EANM (in preparation), EAU (in the past), ESMO, ESO, ESSO	ESOP, EANO, EAPC, OEI, ESTRO, EBMT, EORTC, EUSOMA

EANM: European Association of Nuclear Medicine; EANO: European Association of Neuro-Oncology; EAPC: European Association for Palliative Care; EAU: European Association of Urology; EBMT: European Group for Blood and Marrow Transplantation; ECCO PAC: European Cancer Organisation Patient Advisory Committee; EONS: European Oncology Nursing Society; EORTC: European Organisation for Research and Treatment of Cancer; EPAAC: European Partnership for Action Against Cancer; ESGO: European Society of Gynaecological Oncology; ESMO: European Society for Medical Oncology; ESO: European School of Oncology; ESOP: European Society of Oncology Pharmacy (ESOP); ESSO: European Society of Surgical Oncology; ESTRO: European Society for Radiotherapy and Oncology; EUSOMA: European Society of Breast Cancer Specialists; OEI: Organisation of European Cancer Institutes

ECCO board was written and a presentation on the project was given to the ECCO board on February 12, 2013.

Results

The organisations that returned the questionnaire and participated in the meeting are listed in Table 1.

Multidisciplinary Guideline Development Process

Most organisations involved experts of other disciplines in their guideline groups (EAPC, EAU, EONS, ESMO, ESSO, ESOP, ESO, ESTRO, EANM). However, these experts were not necessarily representatives delegated by an oncology society. It was felt that multidisciplinary guidelines should be developed by representatives of societies.

Furthermore, guideline development should be based on internationally accepted criteria and written by experts in the field of the guideline's topic.

Multidisciplinary guidelines should be developed and evaluated according to existing processes in the different societies (eg, guideline committee) and there should be a transparent conflict-of-interest policy in place.

Partners Involved in Multidisciplinary Guideline Development

The degree of multidisciplinary in the guideline development process should be flexible and adjusted to the topic of the guideline. The group producing a multidisciplinary guideline should involve all relevant disciplines related to the topic. Different guidelines may exist on the same topic with differ-

ent accents depending on the partners involved provided that quality criteria are fulfilled. Patient representatives should be involved in guideline development.

Formats of Multidisciplinary Guidelines

Guidelines can be presented in different forms (eg, extensive version, short version, patient information sheet). Flow charts may add to the user-friendliness and implementation of guidelines. However, it should be avoided that the user only focuses on a specific part of the flow chart. If flow charts are consulted, they should be read completely by the user. It was also felt that when constructing flow charts, professional help may be needed for their development. Advantages of flow charts are that they may serve as quality control tools and may be available in an electronic application.

Quality Control

Guidelines should be of high quality and evaluated according to internationally accepted criteria. They may include quality outcome indicators and measures that can be used by users to evaluate their own performances.

Legal Issues

Implementation of guidelines needs to take into account special local circumstances and patient wishes.

ECCO Quality Criteria

ECCO will endorse multidisciplinary oncology guidelines if they fulfil the following quality criteria:

- Guidelines must be multidisciplinary and must involve representatives of the societies of the relevant disciplines.
- Validated methodologies must be used and must be explicit and transparent.
- A conflict-of-interest policy must be in place and transparent.
- Representatives of patient organisations must be involved.

Role of ECCO in the Guideline Development Process

The role of ECCO was discussed during the forum and at the board. It was concluded that ECCO will not develop clinical practice guidelines. ECCO has a role in

- serving as a switchboard for its members and other European societies to inform about the development of new multidisciplinary clinical cancer guidelines so that all interested societies can be involved. This functionality is offered to ECCO members and other European societies as a way to facilitate collaboration between relevant disciplines and develop multidisciplinary guidelines. This will help avoiding redundancies.
- endorsing multidisciplinary guidelines that fulfil the quality criteria and are submitted by the development group for review.
- disseminating the European multidisciplinary guidelines that have been endorsed. ECCO will create a dedicated webpage for the dissemination and promotion of endorsed guidelines (with links).
- representing the voice of oncology on European oncology guidelines at the EU policy level.

Conclusion

Oncology is multidisciplinary and this should be reflected in multidisciplinary guideline development. In this project, different oncology societies discussed the process, the partners, the format, and quality control of multidisciplinary oncology guidelines. Also, the role of ECCO in relation to multidisciplinary guideline development was clarified.

In the future, ECCO will serve as a switchboard for multidisciplinary oncology guidelines and will promote European oncology guidelines fulfilling the ECCO quality criteria. An action plan is being developed, including standard operating procedures to enable ECCO to fulfil its new role.

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Applications of Multifunctional Nanoparticles in Malignant Brain Tumours

Costas G Hadjipanayis¹, Alexandros Bouras¹, Susan Chang²

Abstract: Malignant brain tumours are one of the most devastating human cancers associated with high mortality and morbidity rates. Clinical management of these tumours remains challenging despite recent advances in current treatment strategies. Difficulties in early detection, local recurrence, and resistance to conventional therapies are the major reasons for failure in malignant brain tumour treatment. Nanoparticles have drawn increased interest in treating malignant

brain tumours due to their potential to act as a vector for brain delivery and to provide tumour-specific detection and treatment. Multitasking nanoparticles can be engineered into a single nanopatform and hold great promise for brain tumour diagnosis and treatment. Currently, magnetic nanoparticles are being used for the imaging and treatment of malignant brain tumours in humans. This review article summarizes different types of multifunctional nanoparticles that have

been used both preclinically and in humans for nanoparticle-based brain tumour imaging and therapy. **Eur Assoc NeuroOncol Mag 2014; 4 (1): 9–15.**

Key words: malignant brain tumours, nanoparticles, drug delivery, magnetic resonance imaging, thermotherapy, theranostics, glioblastoma

■ Introduction

Malignant brain tumours remain a major clinical problem despite improvements in surgery and multimodal adjuvant therapies. Malignant gliomas represent the most common and aggressive malignant brain tumours. The median survival of malignant glioma patients ranges between 3 and 16 months and has virtually remained unchanged during the last 3 decades [1, 2]. Management of malignant gliomas poses a surgical challenge due to their proximity to eloquent anatomical structures within the brain and also their diffuse infiltrative nature which precludes complete surgical resection. The therapy of malignant gliomas is further limited by the inadequate delivery of therapeutic agents to the brain due to the presence of the blood-brain barrier (BBB) as well as non-specificity targeting. The application of novel therapeutic agents for the treatment of malignant brain tumours is urgently needed.

Among different therapeutic approaches, nanotechnology appears to be a promising tool for advancing cancer therapies. Nanotechnology is defined as the manufacturing and construction of materials in the nanometer scale size range of 1–100 nm [3, 4]. Nanomedicine is the use of nanotechnology in medicine and health care [3, 4]. Cancer nanotechnology is the application of nanotechnology toward various aspects of detection, imaging, treatment, and monitoring of cancer [5]. Current innovations in nanotechnology hold great promise in changing the foundations of cancer diagnosis and therapy. The potential application of nanoparticles (NP) to the diagnosis and treatment of malignant brain tumours is now being explored. Various nanotechnology platforms have been used for improving malignant brain tumour imaging, drug delivery to brain tumours, and therapeutic efficacy. One of the most promising aspects of NP-based cancer therapy is multi-

functionality. NPs can be attached to different types of small molecules such as targeting ligands, imaging, and therapeutic agents to serve as diagnostic and therapeutic agents simultaneously [6–12]. The integrated diagnostic and therapeutic capability of nanotechnology has attracted particular attention for diagnosing and treating brain tumours. This review article focuses on different types of multifunctional NPs that have been studied in the management of brain tumours in preclinical models as well as human patients.

■ NPs and Brain Tumours

The unique properties of NPs offer several advantages over conventional malignant brain tumour therapeutic agents. One advantage may be more effective delivery of compounds to the brain tumour site in comparison to conventional drug delivery systems [13–15]. The flow of NPs through blood capillaries and uptake by cancer cells can be facilitated by their small size [13, 14]. The NPs can be engineered to contain small molecules such as contrast agents and drugs [13]. Multiple types of small ligands with different functions, such as MRI contrast enhancement or therapeutic agents, can be incorporated into a nanoparticle and delivered to the brain tumour site. The specificity of the delivery can be achieved by adding targeting ligands, such as monoclonal antibodies, to the nanoparticle surface in order to ensure targeted delivery of the agents to the brain tumour site. The ability of nanopatform-based targeted delivery of imaging or therapeutic regimens to the brain tumour site at effective concentrations is the fundamental principle of multifunctionality of NPs and the key factor for efficient cancer diagnosis and therapy.

Another advantage of nanotechnology compared to conventional imaging and therapeutic agents is the tendency of NPs to accumulate within the brain tumour site via the enhanced permeability and retention effect (EPR). The growth of the tumour results in neoangiogenesis in order to provide cancer cells with oxygen and nutrients for rapid proliferation [16]. The defective architecture of the neovascularization resulting in leaky vasculature, along with the expression of vascular mediators of extravasation (eg, nitric oxide, VEGF) are thought to be responsible for the EPR effect and selective retention of NPs at the brain tumour site [17–19]. In con-

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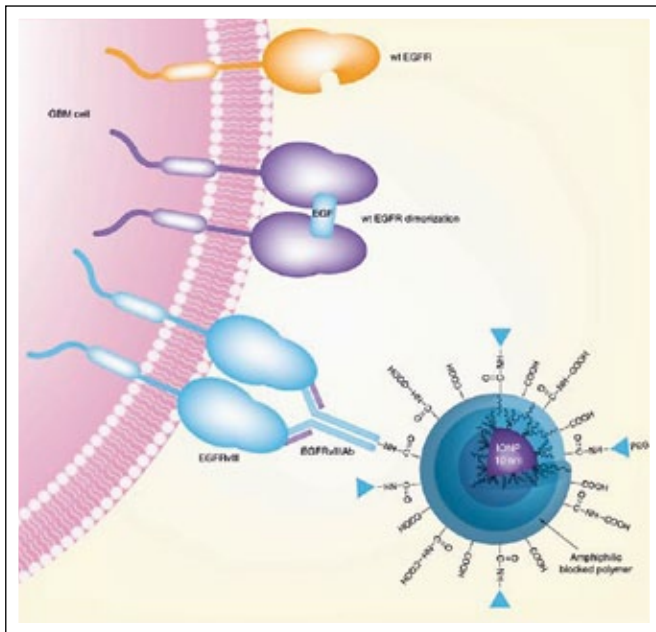


Figure 1. Illustration of an EGF receptor VIII-expressing glioblastoma cell bound by an EGF receptor VIII antibody-conjugated magnetic nanoparticle construct. Reproduced from [Expert Review of Clinical Pharmacology, March 2012, vol 5, no 2, pp 173–86] with permission from Expert Reviews Ltd. The wt EGFR dimerizes upon ligand binding. The truncated EGFRvIII deletion mutant, which does not require a ligand for activation, is bound by an EGFRvIII antibody-conjugated magnetic nanoparticle conjugate (EGFRvIIIAb-iron oxide nanoparticle). The EGFRvIIIAb-iron oxide nanoparticle is comprised of a 10-nm iron oxide core surrounded by an amphiphilic triblock copolymer, which is covalently conjugated to the EGFRvIIIAb.

Ab: antibody; EGFR: EGF receptor; GBM: glioblastoma; IONP: iron oxide nanoparticle; wt: wild-type.

trast, small molecules, such as conventional chemotherapeutic agents, diffuse freely across the tissues and the EPR effect is not observed.

In regards to treatment toxicity, the payload of the NPs can be isolated from the surrounding normal tissues by the addition of biocompatible polymers, preventing the release of the loaded agents within those normal tissues. The result is increased maximum tolerated dose of the therapeutic agent and reduced systemic toxicity. Moreover, targeted delivery of therapeutic agents encapsulated into NPs in conjunction with retention of NPs within the brain tumour site can lead to higher localized concentrations of the agents within the tumour mass, while preventing the undesired systemic consequences of the therapeutic agents [20, 21].

Taking into account the above advantages of nanotechnology, many different types of NPs have been examined in malignant brain tumour research. These include magnetic iron oxide (Fe_3O_4) NPs [22–33], gadolinium (Gd) NPs [34, 35], gold NPs (AuNPs) [36], quantum dots (QDs) [37, 38], dendrimers [39–42], carbon nanotubes (CNTs) [43–45] and polymer-based NPs [46–61].

■ Magnetic NPs

MRI Contrast Effect

Magnetic NPs (MNPs) have been studied as potential diagnostic and therapeutic tools in malignant brain tumours. The unique paramagnetic properties of MNPs enable their detection by magnetic resonance imaging (MRI), and have been

used as both T_1 and T_2 MRI contrast agents [62–65]. Most MNP formulations are comprised of iron-oxide nanoparticles (IONPs). Superparamagnetic iron-oxide NPs (SPIOs) have most commonly been used as MRI contrast agents [66, 67]. Ultrasmall SPIOs (USPIOs) with size < 50 nm have also been examined as potential MRI contrast agents [68]. The MRI contrast effect can occur in both T_2 -weighted MRI sequences, where they produce a hypointense (dark) signal (negative contrast enhancement), and in T_1 -weighted images, where they produce a hyperintense (bright) signal (positive contrast enhancement) [69–71]. The major advantage of USPIOs, compared to conventional Gd- (gadolinium-) based contrast agents, is their prolonged MRI contrast effect due to uptake by tumour cells and microglia (reactive phagocytic cells in the brain) and retention within the brain [72]. Administration of USPIOs leads to an observed peak enhancement for approximately 24 hours that can persist for up to 72 hours in contrast to Gd-based contrast agents which are rapidly cleared by the kidneys [34, 73–75]. In an attempt to overcome the rapid elimination of conventional Gd and increase its retention within brain tumours, Gd NPs have also been reported. Incorporation of Gd into therapeutic NPs has been utilized to track them by using MRI [76]. Functionalized Gd NPs have also been reported as radiosensitizing agents [77]. Gd NPs can be better visualized in T_1 -weighted MRI sequences [35]. SPIOs and USPIOs have been shown to have a relatively safe toxic profile with no evidence of brain toxicity [67, 78]. These agents can be served as an alternative in patients at high risk for Gd-induced nephrogenic systemic fibrosis [79, 80].

Tumour Targeting

MNPs specifically targeted to tumour cells can further increase their imaging benefits by enhancement of their uptake by the targeted tumour cells [81]. Multiple types of compounds, such as peptides and antibodies, have been reported as potential MNP targeting ligands [82].

Chlorotoxin, a peptide derived from scorpion venom, has been described as a targeting motif for brain tumour cells. Chlorotoxin inhibits tumour infiltration by specific binding and inhibition of matrix metalloproteinase-2 (MMP-2), which is over-expressed on the surface of glioma cells and responsible for the degradation of extracellular matrix during tumour invasion [83–85]. Conjugation of chlorotoxin to MNPs has been reported as a method for targeted brain tumour imaging [24] by MRI in addition to inhibition of tumour cell invasion [86]. Furthermore, incorporation of the fluorescent Cy5.5 molecule to the conjugated chlorotoxin-MNPs allows for simultaneous MRI and intraoperative optical imaging [22, 25, 87]. Chlorotoxin-labelled MNPs have also been utilized for targeted gene delivery to glioma cells [32].

Attachment of another small peptide, called F3, to the MNP surface has also been used for the targeting of brain tumours. F3 targets endothelial cells by specific binding to nucleolin, which is over-expressed on the surface of proliferating vascular endothelial cells of tumour-associated vasculature [88]. Intravenous injection of IONPs coated with F3 has provided more persistent and profound MRI contrast enhancement of intracranially implanted rodent tumours compared to identical non-F3-targeted IONPs [48].

Polymer-coated IONPs have been conjugated to a purified antibody that selectively binds to the epidermal growth factor receptor deletion mutant, EGFRvIII (Figure 1), which is a tumour-specific mutation present on the surface of glioblastoma (GBM) cells. The bioconjugated IONPs can provide simultaneous MRI contrast enhancement, as well as targeted therapy of intracranial human GBM xenografts implanted in rodents after convection-enhanced delivery (CED) [26]. Recent toxicity testing of cetuximab-conjugated IONPs has been reported in healthy canines after CED in the brain [89].

Conjugation of a tumour-specific monoclonal antibody known as L6 to IONPs can provide targeted MRI enhancement of the neovasculature of malignant brain tumours after their uptake by tumour cells [90]. Dextran-coated SPIOs functionalized with an antibody against an insulin-like growth factor domain have also been used for targeted MRI and fluorescent imaging of the GBM vasculature [91].

Cytokines can be utilized for the targeted imaging of malignant brain tumours. Gd-containing metallofullerenes functionalized and conjugated to IL-13 peptides have the ability to specifically bind to glioma cells over-expressing the IL-13 receptor providing targeted imaging of these cells in vitro [92].

Neural Stem Cells

MNPs can also be used for tracking stem cell tropism to malignant gliomas in vivo. Neural stem cells are brain tumour targeting tools as they exhibit tropism for GBM tumours after intracranial administration [93]. This striking characteristic makes neural stem cells a potential candidate for tumour-targeted gene delivery and therapy [94, 95]. IOPNs can label neural stem cells enabling visualization of their migration into the brain [96, 97].

Chemotherapeutics and MNPs

MNPs can also be utilized to deliver chemotherapeutic agents to the brain tumour site while simultaneously tracking them by MRI. IONPs coated with a polymer have been used for both delivery of the chemotherapeutic agent epirubicin and monitoring of their distribution in vivo by MRI [29]. Paclitaxel-loaded MNPs have been utilized to deliver the chemotherapeutic agent paclitaxel in a rat glioma model. Increased drug uptake by the brain tumour cells resulted in enhanced therapeutic efficacy and effective MRI contrast enhancement [30].

Magnetic Targeting and Focal Ultrasound

In an attempt to enhance the delivery of MNPs to malignant brain tumours after systemic administration, magnetic targeting has been described. Magnetic targeting is the application of a magnetic field to enhance accumulation of MNPs within the brain tumour site [98–100]. Another concept that has been described in an effort to increase the systemic delivery and deposition of MNPs into malignant brain tumours is disruption of the BBB. Many strategies have been examined in order to facilitate opening of the BBB. Focal ultrasound is a non-invasive technique that can be utilized for selective BBB disruption in a targeted brain region [101–103]. The synergistic effect of focal ultrasound and magnetic targeting has been demonstrated in a study where both systemic delivery and deposi-

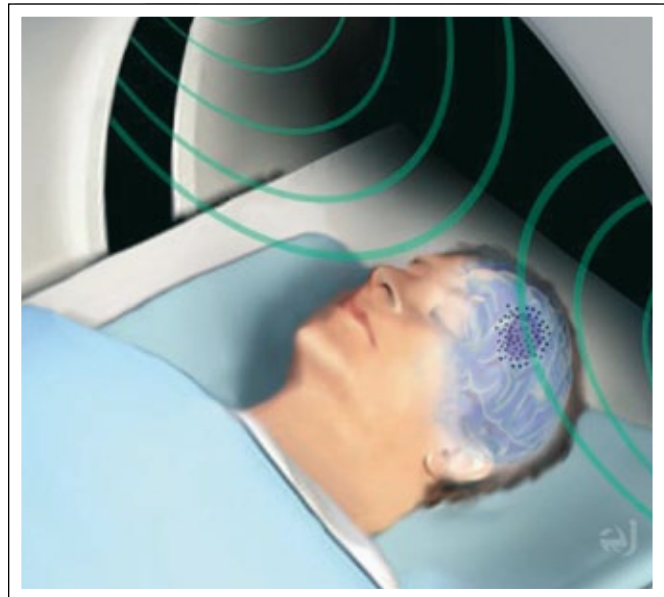


Figure 2. Intratumoural thermotherapy of a malignant brain tumour with magnetic nanoparticles. Reproduced from [Expert Review of Clinical Pharmacology, March 2012, vol 5, no 2, pp 173–86] with permission from Expert Reviews Ltd. A patient who has undergone intratumoural implantation of magnetic nanoparticles is depicted undergoing an alternating magnetic field session for treatment of his malignant brain tumour by thermotherapy.

tion of epirubicin-loaded MNPs into tumour-bearing animals were significantly increased [29].

Thermotherapy

Another function of MNPs is their ability to produce heat with the application of an alternating magnetic field (AMF). Use of hyperthermia for the treatment of cancer, known as thermotherapy, has been demonstrated in patients with human GBM [104, 105] (Figure 2). Temperatures above 41 °C cause heat stress with resultant protein denaturation and DNA cross-linking within the cell, leading to cell apoptosis [106]. Alterations of the tumour microenvironment can also occur [107]. The above changes have a synergistic effect when combined with chemotherapy and radiation [108]. Engineering of MNPs plays a crucial role in maximizing the hyperthermia response [109, 110]. MNPs that can be used for thermotherapy are combinations of various metals such as manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), zinc (Zn), and magnesium (Mg) [111–114]. Iron oxide-based MNPs have been extensively examined for thermotherapy application in brain tumours due to their biocompatibility and safe toxicity profile [115, 116]. Animal models and human patients with malignant brain tumours have been used to evaluate feasibility and safety of MNP-based thermotherapy [104, 105, 117]. Safety and efficacy have been demonstrated holding great promise of this cancer treatment modality.

■ Polymer-Based NPs

A vast amount of research has been made exploring polymer-based NPs in brain tumour diagnosis and therapy. Varying core polymer compositions have been examined with the most popular being the polysorbate 80-coated poly(butyl cyanoacrylate) and poly(ε caprolactone) [46, 50, 51, 53, 54]. Drug delivery to brain tumours is the main application of polymer-based NPs

with doxorubicin, paclitaxel, and camptothecin being used [46, 50, 51, 53, 57, 58, 118]. Polymer-based NPs have been used in clinical trials as vehicles for drug delivery, but the results were discouraging as 1 % or even less of the injected dose was delivered to the brain with most of the nanoparticle-drug conjugate being trapped in the liver [119]. The incorporation of a small peptide sequence known as angiopep has been used for improved targeted delivery to brain tumours [55]. Dual targeting by using 2 different moieties has also been reported. Incorporation of both a small peptide accounting for BBB targeting and an additional small aptamer targeting cancer cells onto a polymer-based nanoparticle has been described [59]. Dual targeting resulted in enhanced tumour distribution of the dual targeted NPs [59]. Furthermore, polymer-based NPs have been successfully used for gene therapy of brain tumours [49, 120]. In one study, incorporation of the integrin-binding motif RDG into a polymer-based nanoparticle enabled efficient targeted delivery of a plasmid expressing an apoptosis ligand in a rat glioma model resulting in increased survival [120]. Moreover, polymer-based NPs have also been utilized for both MRI and drug delivery by loading of both a magnetic contrast agent and a fluorescent drug [48]. Polymer-based NPs can also be encapsulated by both iron oxide and photodynamic therapy (PDT) drugs for both MRI contrast enhancement and PDT of brain tumours, respectively [48].

■ Gold NPs

Gold NPs comprised of a silica core and coated with a gold shell have received increased attention as potential vehicles for delivery of therapeutic agents to the brain, as well as for imaging. Their small size, surface chemistry available for functionalization, and biocompatibility make them strong candidates for biological and medical applications [36, 121–125]. Gold NPs with sizes up to 50 nm are able to cross through the disrupted blood-brain tumour barrier [121, 123, 124, 126]. Gold NPs have been utilized to deliver gadolinium for enabling preoperative detection and surgical planning through MRI, as well as to simultaneously deliver photoacoustic and Raman imaging agents for tumour margin delineation during surgery [127]. Fluorescent imaging agents can also be incorporated into gold NPs for purely diagnostic purposes [36]. Another application of gold NPs is phototherapy. Gold NPs can be designed as nanoshells enabling light absorption in the near-infrared range of the light spectrum which has minimal absorption by water, thus allowing for passage deep into tissues with minimal energy loss. Design of these gold NPs has been achieved and their activation by light has enabled killing of medulloblastoma and glioma cells *in vitro* by phototherapy [128]. Loading of gold nanoshells into macrophages can lead to efficient delivery of the NPs to gliomas and their subsequent activation by near infrared light can result in growth inhibition [129].

■ Quantum Dots

QDs are nanocrystals made of semiconductors with unique optical and electronic properties [130]. They are advantageous for *in vivo* imaging due to the fact that they can emit fluorescence light from 400–2000 nm. Their increased fluorescence emission spectrum allows for enhanced brightness and simul-

taneous multicolour detection [131]. Due to their heavy metal content, toxicity issues are a concern with normal surrounding tissues [131]. QDs can be loaded with contrast agents, such as gadolinium, in order to provide further imaging capabilities in addition to inherent fluorescent properties [37, 132]. However, the above capabilities have not been applied to brain tumours at this point. QDs have also been used for targeted delivery of siRNA for selective inhibition of EGFRvIII expression in human GBM cells [38]. Conjugation of epidermal growth factor (EGF) or EGF receptor antibody to QDs has been attempted and has led to successful specific labelling of human glioma cells *in vitro*, glioma mouse models, and in human brain tumour biopsies by the fluorescent emission of QDs [133].

■ Dendrimers

Dendrimers have been examined as NPs with the potential of delivering agents to brain tumours by crossing the BBB. It has been shown that dendrimers with size < 20 nm can cross the BBB [41]. Dendrimers are spherical molecules formed from repetitively monomeric or oligomeric organic molecule branching units. Their structure, specifically the degree of branching, allows for encapsulation of molecules in the interior as well as on the surface [39, 40, 42]. Dual targeting can be performed with dendrimers [40, 134]. A dendrimer-based nanoprobe has been labelled with both angiopep-2, for higher BBB transcytosis efficacy, and RGD peptides for targeting of the brain tumour vasculature [40]. The near-infrared fluorophore Cy5.5 and rhodamine were added to the dendrimer in order to create a multifunctional nanoprobe allowing for non-invasive preoperative localization of brain tumours, as well as possible intraoperative image-guided tumour resection [40]. A tolerable toxicity after using dendrimers has been reported [39, 40].

■ Carbon Nanotubes

CNTs are formed of graphite sheets assuming a cylinder-shaped configuration. They possess electrical properties and heat conductivity [135]. They have been used as nanovectors for targeted drug and gene delivery into tumours. CNTs can be packaged with siRNA molecules for targeting tumour cells by exerting RNA interference on target gene expression and suppressing tumour growth [136]. CNTs can potentially be used as a nanovector delivery system for targeted delivery of agents into phagocytic cells *in vivo* for modulating macrophage function in brain tumours. Selective uptake by tumour macrophages labelled with non-toxic hydrophobic fluorescent dye multi-walled CNTs has been shown in a murine glioma model [45]. In another study, conjugation of an immunopotent oligodeoxynucleotide to single-walled CNTs has been described. Enhanced targeted delivery and uptake of the immunopotent compound by glioma cells both *in vitro* and *in vivo* has been demonstrated resulting in potentiation of anti-glioma immunity and inhibition of glioma tumour growth [44]. CNTs can also be utilized for thermal ablation therapy. Photothermolysis of GBM stem-like cells by CNTs targeted with CD133 monoclonal antibody has been reported [43]. Both GBM-CD133⁺ and GBM-CD133⁻ cells were treated *in vitro* with single-walled CNTs functionalized with a CD133 monoclonal antibody (CD133Ab-CNT) and subsequent irradiation with near-infrared laser light. The GBM-CD133⁺

cells were selectively targeted and eradicated, whereas GBM-CD133⁺ cells were unaffected [43]. Moreover, GBM-CD133⁺ cells pre-treated in vitro with the CD133Ab-CNTs were injected subcutaneously into mice and then near-infrared laser-induced photothermal ablation was applied. Significant inhibition of both tumour growth rate and tumour progression was observed [43]. This study has demonstrated the potential utilization of CNTs as a thermal-coupling agent for effective targeting and inhibition of growth of GBM stem-like cells.

■ Human Clinical Translation of NPs

Although the benefits of NPs in cancer diagnosis and therapy have been widely explored, their potential effects in humans are still unclear. The translation of NPs into clinical use remains challenging. Various issues, such as pharmacokinetics, biodistribution, side effects, potential toxicity, and the immune system reaction to NPs, remain to be addressed and are of great importance in order to establish whether and which NPs can be used in humans. The recent advances of cancer nanotechnology are necessary to proceed in parallel with bioactivity and toxicity assessment studies before clinical application. Numerous nanoparticle platforms are currently under different stages of preclinical and clinical development. However, in the brain tumour field, investigation of potential clinical use of NPs remains limited. MNPs, specifically IONPs, are the most advanced NPs in terms of translation into clinical application for brain tumours. Ferumoxytol, a USPIO that targets phagocytic cells, has been used in the imaging of patients with malignant brain tumours. Patients underwent serial MRI up to 72 hours after a single dose of ferumoxytol and the time course of enhancement was compared with baseline Gd scan [73]. Maximal ferumoxytol-induced enhancement intensity was observed at 24–28 hours after administration and enhancement was expanded into non-Gd enhancing regions of infiltrating brain [73]. The use of IONPs as MRI contrast agents can provide visualization of brain tumours that is not apparent with conventional Gd. Furthermore, the delayed peak enhancement provided after IONP administration compared to Gd can allow for assessment of postoperative residual tumour without the need of re-administration of a contrast agent. In another study, intraoperative MRI (iMRI) with ferumoxtran-10, also a USPIO, was used in patients who underwent malignant brain tumour surgical resection [137]. Less confounding (non-tumoural) contrast enhancement was observed during iMRI compared to iMRI after Gd administration [137]. IONPs have also been used for hyperthermia-induced tumour ablation, known as thermotherapy, in patients with recurrent GBM. In a phase-II clinical trial, aminosaline-coated IONPs have been used for hyperthermia induction after intratumoural injection in patients with recurrent GBM and application of AMF in combination with fractionated external beam radiotherapy (EBRT) [104, 105]. Direct inoculation of IONPs into tumours using stereotactic-guided injections was used, followed by multiple thermotherapy sessions, demonstrating safety and efficacy in combination with EBRT [104, 105].

■ Conclusion

Effective treatment of malignant brain tumours poses a significant challenge. Recent advances in microsurgery and mul-

tifunctional adjuvant therapy have only resulted in a modest improvement in patient prognosis. Novel technologies are therefore needed to be applied to the management of malignant brain tumours. Nanotechnology has quickly emerged as a promising tool having the potential to change multiple aspects of malignant brain tumour diagnosis and treatment. Several types of NPs have been described providing MRI contrast enhancement, intra-operative tumour delineation, and targeted delivery of chemotherapy or gene therapy, as well as thermotherapy. Currently, NPs are being used in humans for imaging and thermotherapy of malignant brain tumours. Multifunctional NPs, which have the potential for simultaneous targeted cancer cell delivery, imaging, and therapy, form the basis for new approaches combating malignant brain tumours. Additional improvements in the design and surface chemistry of NPs will permit better delivery and penetration within brain tumours. The comprehensive assessment of the toxicological effects of NPs remain to be further determined in the future management of brain tumour patients.

■ Conflict of Interest

The authors report no conflict of interest with the material presented in this manuscript.

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Reoperation for Recurrent Glioblastoma: Outcome Analysis and Correlation with MGMT Status

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Abstract: The treatment of recurrent glioblastoma remains a challenge. Although second surgery may provide effective palliation, it has yet to be established whether it has a role at all. The few studies investigating this issue are small, retrospective, and characterized by inhomogeneous datasets.

Moreover, the role of MGMT at the time of relapse remains controversial, since MGMT status may vary across the disease's natural history and its impact on post-progression survival is unclear.

The aim of this study was therefore to analyze predictors of outcome in patients with recurrent glioblastoma and to compile a review of

data in the literature with a view to comparing the effect on outcome of second surgery against well-known prognostic determinants. **Eur Assoc NeuroOncol Mag 2014; 4 (1): 16–9.**

Key words: glioblastoma, recurrence, second surgery, prognostic factors, MGMT methylation

■ Introduction

Although we have improved our understanding of glioblastoma biology and achieved an improvement in survival thanks to the use of combined radio- and chemotherapies [1], many treated patients relapse and their prognosis is extremely poor. Following standard treatment, the 5-year survival rate amounts to approximately 10 % [2]. The therapeutic options available for patients with disease progression are compromised because the efficacy of chemotherapy is limited and neurologic deterioration is often severe; approaches for those with recurrence include second surgery, usually considered indicated since it can relieve symptoms. Few retrospective studies have been conducted to ascertain the outcome of recurrent glioma patients who undergo second surgery for relapse.

■ Prognostic Factors and Second Surgery

The only prospective data regarding outcome of patients who have undergone surgery for recurrent disease comes from the phase-III trial that randomized patients with gliomas (65 % glioblastomas) to receive surgery with carmustine implants or surgery with placebo at relapse [3]. In this trial, patients who underwent surgical resection with placebo showed a median survival of about 5 months. Factors that were significant predictors of outcome in patients with glioblastoma included age ($p = 0.004$), interval from previous surgery ($p < 0.001$), Karnofsky Performance Status (KPS; $p = 0.02$), race ($p = 0.06$), and previous nitrosourea chemotherapy ($p = 0.03$).

Retrospective Studies

Moreover, many retrospective data are available (Table 1). The first important reports [4, 8, 14] on this issue appeared in 1987, when Ammirati studied 55 cases of recurrent anaplastic astrocytoma and glioblastoma (20 and 35, respectively); in all cases, second surgery was performed; 41 of the 55 patients (75 %) underwent postoperative chemotherapy and/or radio-

therapy. Overall median survival was 92 weeks, whereas median survival time after re-surgery was 36 weeks. Outcome was better in patients with low-grade recurrence at histology and a good KPS (> 70) who underwent radical resection. At multivariate analysis, the most powerful variables were KPS and extent of resection. However, the reliability of the data reported in this study, one of the first to suggest a correlation between reoperation and survival, was undermined by the fact that it was conducted on a small series of patients with different histologies. Landy [5] analyzed 33 recurrent glioma patients, all of whom had second surgery. After surgery, 9 patients had re-irradiation and 24 received chemotherapy. Median survival after reoperation was 8 months, 13 months, 22 months, and 47 months for glioblastoma, anaplastic astrocytoma, astrocytoma, and oligodendroglioma/mixed group, respectively. Despite a trend toward improvement in survival depending on the tumour grades, the difference did not attain statistical significance; a significant correlation was found between age/KPS and prognosis, with younger patients with a better KPS having a longer survival. According to the authors, 11 patients benefited from second surgery and any consideration of aggressive treatment for recurrent gliomas should be balanced against the predicted outcome since symptoms of pressure, unlike those of infiltration, are usually ameliorated by surgery. The small series precluded any sound conclusion: patients with a better histological diagnosis would probably have enjoyed a better outcome irrespective of the surgical approach.

Keles reported on a series of 92 patients with hemispheric glioblastoma who underwent a total of 107 operations [6]; 52 were undertaken on patients who had undergone previous surgery or biopsy; the percentage of resection (POR) and volume of residual disease (VRD) were calculated using volumetric image technique analysis. Preoperative KPS, chemotherapy, POR, and VRD have a statistically significant effect on time to tumour progression: improvement in outcome was proportionate to residual disease reduction.

Pinsker reported on 38 patients who underwent repeat surgery for recurrent glioblastoma [6], survival was longer in patients in whom total resection was achieved than in subtotal resection (21 and 18 weeks, respectively), although the difference was not of statistical significance. Moreover, on eval-

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Table 1. Studies.

Author, year [Ref]	n	Setting (newly/recurrent)	Histology	Role of surgery
Ammirati, 1987 [4]	55	Recurrent	GBM, AA	Positive
Landy, 1994 [5]	33	Recurrent	GBM, AA, WHO grade II	ns
Keles, 1999 [6]	92	Mixed (48 % second surgeries)	GBM	Positive
Pinsker, 2002 [7]	38	Recurrent	GBM	ns
Mandl, 2008 [8]	32	Recurrent	GBM	Negative
McGirt, 2009 [9]	949	Mixed (73 % second surgeries)	GBM, WHO grade III	Positive
Clarke, 2011 [10]	758	Recurrent	GBM	ns
Carson, 2007 [11]	333	Recurrent	GBM, WHO grade III	ns
Gorlia, 2012 [12]	300	Recurrent	GBM	ns
De Bonis, 2012 [13]	76	Recurrent	GBM	Positive

ns: not significant; GBM: glioblastoma; AA: anaplastic astrocytoma

uating the effect of conventional variables (age < 50 years, time between first and second resection > 26 weeks, KPS > 90 at second resection, total primary resection) they found a correlation with longer survival; for postoperative survival, extent of resection, unlike age and recurrence-free interval, KPS was predictive of outcome improvement. This data suggests that second surgery enhances efficacy of treatment in patients with a good KPS. Although this study is valuable because it was performed on a homogeneous, well-defined, and stratified cohort, the size of the series compromises the value of the evidence provided.

In Mandl's retrospective analysis of 32 patients with recurrent glioblastoma [8], the inclusion criteria were good clinical condition (KPS at least 60), local recurrence without multifocality, and the feasibility of debulking. The cohort was split into 3 subgroups: 9 patients received only surgical resection as salvage therapy (4 had > 1 resection), 11 had surgery plus chemotherapy or stereotactic radiosurgery (SRS), and 12 were given chemotherapy alone or SRS. Median overall survival was 34 weeks in patients who had chemotherapy or SRS plus surgery, 28 weeks for those given chemotherapy alone or SRS, and 13 weeks for those resected without further treatment. The authors therefore advised that patients with severe mass effect symptoms be considered candidates for resection followed by further salvage therapy but that patients with mild symptoms could be spared surgery since a similar survival advantage can be gained by means of other approaches. Importantly, in 40 % of re-operated patients, the performance status deteriorated; this suggests that second surgery may be detrimental.

McGirt [9] made a retrospective analysis on a large series of patients with malignant astrocytoma; altogether 949 patients were considered: 700 were WHO grade IV and 249 WHO grade III; 549 had primary resection and 400 second surgery; 294 of the latter had glioblastoma. Resection was considered gross-total (GTR) in the absence of contrast enhancement at postoperative MRI, near-total (NTR) if a rim enhancement of the resection cavity was evidenced, and sub-total (STR) if nodular enhancement was found. In patients given second surgery, median survival after GTR, NTR, and STR was 11, 9, and 5 months, respectively. At adjustment for variables independent at multivariate analysis (ie, age, KPS, and temozolomide adjuvant therapy), GTR and NTR were associated with improved survival; GTR provided a 10-% greater reduction

of the risk of death than NTR, which provided a further 37-% greater decrease than STR. The time interval between 2 subsequent surgeries and the tumour site was not a significant factor. The author concluded that extensive resection, even at the time of recurrence, may provide better outcome and patients receiving optimal surgery are more likely to benefit from subsequent therapy. This study is significant because the series was large and any bias obviated by precautions such as a blind review of the neuro-radiological images.

Park [15] evaluated 34 consecutive patients with recurrent supratentorial hemispheric glioblastoma to identify a prognostic model predictive of outcome. Patients had radical resection for recurrence in a single institution with all procedures being performed by the same surgeon. At survival analysis, significant variables for survival were KPS < 80, tumour mass > 50 cm³, and a motor-speech-middle (MSM; a scale to assess the involvement of 3 eloquent/critical brain areas) cerebral artery score > 2. The findings from these parameters were used to determine the overall score for each case and patients were subsequently divided into 3 prognostic groups; median survival was 10.8 months for patients with null scores and 1 month for those with 3.

Commenting on this study, Xu et al [16] argued that since the number of patients believed to have the worst prognosis was only 10 % there might have been a selection bias precluding a reliable conclusion; moreover, they stated that the performance status alone cannot be considered an independent prognostic factor because it is frequently influenced by tumour location; finally they pointed out that although all patients underwent surgery some might have benefited from upfront chemotherapy without any adverse effect on the final outcome.

Clarke [10] analyzed 758 patients with recurrence from glioblastoma; of the cohort enrolled in the North American Brain Tumor Consortium (NABTC) phase-II clinical trials, 208 underwent second surgery at the time of disease progression/relapse. Patients who underwent surgery were compared with those who did not for progression-free survival at 6 months (PFS6) and overall survival. No difference was found between the surgical and non-surgical groups, either for progression-free and overall survival, which ranged from 8–19 weeks and from 24–34 weeks, respectively. Chamberlain and Silbergeld [17] questioned the methodological approach used in this

study, arguing that it did not report data on tumour volume or extent of resection; nor did the authors evaluate whether there was any correlation between surgery and improvement of symptoms – this would have strengthened their assumption that second surgery does not affect outcome.

De Bonis [13] made a retrospective evaluation of 76 recurrent glioma patients, 17 of whom had second surgery alone, 24 chemotherapy alone, 16 surgery and chemotherapy, and 19 no treatment; it was found that patients undergoing surgery and chemotherapy lived longer than patients who had alternative treatment. Moreover, unlike age and extent of resection, performance status was a significant independent prognostic factor.

Carson et al [11] reported the results of a recursive partitioning analysis (RPA) conducted on 333 recurrent glioma patients in phase-I and -II trials in order to investigate systemic or local chemotherapy and brachytherapy in the New Approaches to Brain Tumor Therapy (NABTT) CNS Consortium. Glioblastoma patients made up 67.4 % of the study population; 44.5 % (n = 146) underwent second surgery. The RPA selected different prognostic factors for the glioblastoma and non-glioblastoma groups. The former was split on the basis of the Karnofsky Performance Status (60–70 vs 80–100), age (≥ 50 vs < 50), and tumour location (outside frontal lobe versus confined to frontal lobe) while the latter was split on the basis of the Karnofsky Performance Status (60–80 vs 90–100), age (≥ 50 vs < 50), and corticosteroid use. Seven classes were identified during analysis. Survival ranged from 3.8 months for patients with a histology other than glioblastoma and a low KPS via 10.4 months for glioblastoma patients < 50 years and a high KPS to 25.7 months for non-glioblastoma patients with a frontal tumour and good performance status. The number of surgical procedures performed was not significant at multivariate analysis being ruled out by RPA.

To investigate prognostic factors in recurrent glioma patients Gorlia [12] considered 300 patients recruited in phase-I or -II trials conducted by the EORTC Brain Tumor Group: 138 had received temozolomide concomitant with and adjuvant to radiotherapy, 158 radiotherapy alone or combined with another chemotherapy regimen, and 4 chemotherapy without previous radiotherapy. Post-progression survival and the progression-free survival were 6.2 months and 1.8 months, respectively. The prognostic variables that proved significant were PS, neurological deficit, time since primary diagnosis, baseline administration of steroids, number of target lesions, tumour size, frontal tumour location, and prior chemotherapy with temozolomide; at multivariate analysis, variables impacting on overall survival were performance status, baseline steroids, number of target lesions, frontal location, and tumour diameter; age and second surgery at recurrence did not affect survival. It should be pointed out that only 8 % of patients underwent second surgery.

MGMT Methylation

The value of tissue in recurrence from glioblastoma is questionable; however, it is probably advisable to achieve the best possible definition of the biological signature at recurrence,

although predictive markers for decision-making after reoperation are lacking. MGMT methylation status at the time of first surgery has been shown to be a potent prognostic factor for patients treated with either RT followed by temozolomide or temozolomide concurrent with and adjuvant to RT [18]. However, it is unclear whether this epigenetic feature is consistent also at the time of disease recurrence after postsurgical radiotherapy (RT) followed by temozolomide and whether its prognostic role is retained. In a consecutive and prospectively recorded database of glioblastoma cases, Brandes et al [19] evaluated data from tumour specimens obtained during first and second surgery. Concordance between MGMT methylation status at the time of first and second surgeries was relatively low (63 %). Moreover, MGMT status changed more frequently in patients with MGMT-methylated (61.5 %) than in patients with unmethylated (24 %) status at first surgery ($p = 0.03$). Interestingly, patients treated with concurrent chemotherapy/RT were characterized by a substantially high percentage of MGMT shifts from methylated status at the time of first surgery to unmethylated status at the time of second surgery ($p = 0.03$).

On the contrary, another retrospective series [20] showed concordance between first and second evaluation of the MGMT methylation status in 89 % of glioblastoma patients. In this study, MGMT methylation was associated with longer progression-free survival, overall survival, and post-progression survival.

Discussion

Age and KPS are valid prognostic factors. Furthermore, the majority of studies suggested tumour size as a valuable predictor of outcome. Conflicting data are available regarding the role of tumour site as a factor affecting survival since in some studies tumour involvement of eloquent brain areas is associated with poorer outcome. However, surgery did not prove significant in the study by Clarke, who provided a valuable analysis conducted on a large series.

The role of MGMT methylation status as a prognostic factor for post-progression survival remains unclear and warrants further studies.

As yet, there is little consensus regarding the role of surgery for recurrent glioblastoma, common limitations are the small patient series and heterogeneity in samples; treatment options at recurrence can vary, often depending on the urgent need to alleviate symptoms, and taking previous treatment and performance status into account. Finally, age and KPS were the only true predictors of survival, proving significant in all the studies reviewed. Since a trial randomizing patients to surgery versus chemotherapy is not feasible from an ethical standpoint, an analysis on a large series of patients using prospectively collected data would be welcome since it would provide a more reliable insight on the value of second operation for patients with recurrent glioblastoma.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Optical Biochemical Imaging: Potential New Applications in Neuro-Oncology

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Abstract: For the surgical treatment of malignant gliomas and adjustment of adjuvant therapies it is crucial to characterize the tumour as precisely as possible. This includes the determination of the exact tumour location as well as the analysis of its properties in order to define an accurate diagnosis as early as possible in the treatment process. New, purely optical, non-invasive techniques allow for the label-free analysis of tissue and are promising for neurosurgical applications. These techniques may be helpful for neuropathology and have the potential to be used for intraoperative *in situ* diagnosis of suspicious areas without

the need for removal biopsies. The ability of linear (Fourier-transform infrared, Raman [FT-IR], Raman), and non-linear (coherent anti-Stokes Raman scattering [CARS], surface-enhanced Raman scattering [SERS]), optical, vibrational spectroscopy, also in combination with multiphoton technologies (second harmonic generation [SHG], two-photon-excited fluorescence [TPEF]), to characterize brain tumours has already been shown in animal models of experimental glioma and on different human brain tumour entities. Based on biochemical composition, glioma tumours can be identified and characterized, the borders towards normal

brain tissue and infiltrative areas can be discerned with cellular resolution and morphological details help to identify functional fibre tracts. Label-free, optical biochemical imaging technologies can provide clinically relevant information and need to be further exploited to develop a safe and easy-to-use technology for intraoperative *in situ* diagnosis of malignant glioma. **Eur Assoc NeuroOncol Mag 2014; 4 (1): 20–6.**

Key words: brain tumours, intraoperative imaging, vibrational spectroscopy, coherent anti-Stokes Raman scattering (CARS)

■ Introduction

Malignant gliomas are a severe and life-threatening pathology because of their invading properties and limited intracranial space. To increase the probability of a positive outcome and to provide optimal, personalized therapy, it is crucial to precisely determine tumour localization and boundaries as well as the tumour's exact properties and characteristics. This information is required in the clinical time course as early as possible to adjust therapy accordingly.

Gliomas are typically resected by surgery followed by the application of adjuvant therapies, ie, irradiation and chemotherapy [1]. It is evident that especially high-grade malignant gliomas have to be removed as completely as possible while preserving the surrounding functional brain [2]. Preoperative MRI images provide information about the localization and size of the tumour. These images can be used for neuronavigation during surgery but they cannot compensate for intraoperative tissue changes and alterations, eg, shifts [1]. Intraoperative MRI requires profound constructional changes of the operating theatre, and special, expensive equipment [3]; it is time-consuming (15–30 min) and is used to optimize the extent of resection [4], ie, for resection control and not during ongoing surgery. Information about localization of certain tumour types can also be obtained by fluorescence monitoring: glioblastoma multiforme, the most malignant brain tumour in adults, accumulates 5-aminolevulinic acid and can be visualized intraoperatively by the fluorescence of the resulting me-

tabolites [5]. Other fluorescence dyes take advantage of tumour vessel depiction (indocyanine green) or of vascular leakage into the neoplastic tissue (fluorescein) and may be suitable for various brain tumour types, but none is commonly used for tumour resection [6]. The information obtained by intraoperative administration of fluorophores or contrast agents can be improved by the application of advanced optics like confocal or multiphoton technologies that permit to visualize fine structural details like cytoarchitecture [7]. Nevertheless, there is no method available that allows the localization of every type of brain tumour during surgery. The exact delineation between normal and tumorous tissue during surgery is still an unsolved problem. Additionally, it is not possible to detect small micrometastases before the breakdown of the blood-brain barrier with the technology clinically available [8, 9].

To optimize the resection strategy and decide upon radical resection of aggressive and highly malignant glioma, histopathological analyses are performed during surgery. Therefore, a biopsy sample of suspicious tissue is removed, tissue smears or sections are prepared and stained, and the tissue morphology is evaluated by a trained pathologist [10]. However, this method is time-consuming (approximately 30 min) and of a retrospective nature, ie only applicable after removal of the tissue, and does not allow for precise cancer recognition in those cases when representative specimens cannot be obtained. On the other hand, neurosurgery renders it possible to visually access the tumour and therefore theoretically opens the possibility to perform *in situ* diagnosis without tissue removal by applying non-invasive optical analysis of suspicious tissue.

Microscopy is a well-established technique to assess morphology of cells and tissue and to research and diagnose diseases of all kinds. Usually, special dyes are required to generate contrast in the substantially transparent tissue. There are a variety of dyes to visualize certain biochemical compounds or classes of compounds, ie classical stains discern basic or acidic compounds or antibodies are coupled to certain fluorophores or are visualized by chemical dye reactions

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and address very specifically markers on the cell surface or within the cells. Other dyes can be used to follow tracts and emphasize structural differences. However, most dyes are not approved for clinical use. New advanced optical methods allow the label-free investigation of tissues and have the potential for histopathological diagnosis [11] and even intravital application, a property that makes them useful in neurosurgery. They address biochemical properties (vibrational spectroscopy [12], coherent anti-Stokes Raman scattering [CARS], two photon-excited fluorescence [TPEF], second harmonic generation [SHG] [13]) and, to a certain extent, also morphological properties (CARS, TPEF, SHG) while other techniques focus on physical properties of the tissue (optical coherence tomography [14], intraoperative optical imaging [15]). Therefore, the present research focuses on the development of advanced, label-free imaging techniques for brain cancer pathology assessment and new tools for *in vivo* identification of glioma margins.

We present an overview of new biochemical imaging techniques that are already in use for experimental brain tumour research (vibrational spectroscopy, CARS, TPEF, SHG) and have the potential to be integrated in routine procedures during treatment and diagnosis of human malignant glioma [16].

Vibrational Spectroscopy

Vibrational optical spectroscopy comprises a series of different methods. In contrast to conventional staining methods they offer overall molecular specificity and utilize the interaction of electromagnetic radiation with the sample constituents to reveal chemical composition [17].

Specific vibrations of chemical bonds within the sample lead to characteristic alterations in electromagnetic radiation used for excitation and are visualized by a specific pattern of bands in the corresponding spectra (Figure 1). In case of biological samples and tissues, which consist of a variety of different biochemical compounds, vibrational spectra are the products of the complex overlap of multiple bands of the chemical bonds of all tissue constituents. Therefore, vibrational spectra comprise the entire information about cell or tissue biochemistry and are referred to as biochemical fingerprint.

Besides the identification of chemical compounds, also quantitative information is provided by vibrational spectroscopy. Changes in band amplitude are proportional to the concentration of a particular compound or functional group. Consequently, the spectra of biological specimens reflect both the structural complexity of the individual components and their relative abundances. Vibrational spectroscopy is regarded as the analytical method with the highest density of information.

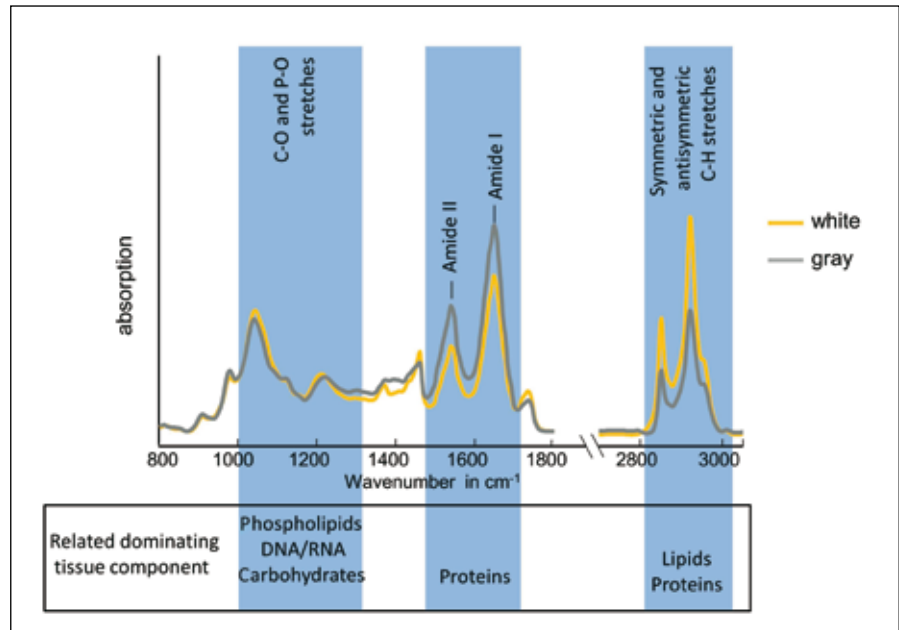


Figure 1. FT-IR spectra of grey and white matters of nervous tissue. The main bond vibrations and related dominating tissue components are indicated.

Spectroscopic information of several positions of a sample can be used to build images that visualize the underlying biochemical composition. Each point of the spatially resolved data set, the hyperspectral cube, contains the information of one spectrum. Usually, the application of advanced chemometric tools is required to extract clinically relevant features from the spectral datasets that can be displayed in different types of spectroscopic images [18]. Therefore, advances and research of new applications go hand in hand with advances in computer power and speed that are necessary to handle and analyze large datasets. Vibrational spectroscopic techniques therefore have been gaining increasing attention for biomedical applications, investigation of disease mechanisms, and diagnosis over the past years [17, 19].

Fourier-Transform Infrared Spectroscopy

Fourier-transform infrared spectroscopy (FT-IR) is based on the absorption of infrared radiation by the sample and its use in biomedical science has been studied for many decades. Starting with the investigation of single compounds, technical progress and advances in computational science permitted to analyze more complex tissue samples and to apply sophisticated analytical methods to large datasets. The entire compositional information about the biochemistry of nervous tissue contributes to FT-IR spectra. Figure 1 shows the typical FT-IR spectrum of grey and white matter. Bands in the region of 1000–1350 cm^{-1} are dominated by the vibrations of phosphate groups and carbohydrates and indicate mainly the presence of phospholipids, DNA/RNA, and carbohydrates in the context of cells and tissue [20]. Prominent bands of amide II and amide I bond vibrations of proteins are recognized at around 1550 and 1650 cm^{-1} , respectively. In the high-energy region from 2800–3000 cm^{-1} , bands related to C-H bond vibrations in lipids and proteins are found. Applied as an imaging technique FT-IR provides spatially resolved information about distribution of tissue constituents (Figure 2).

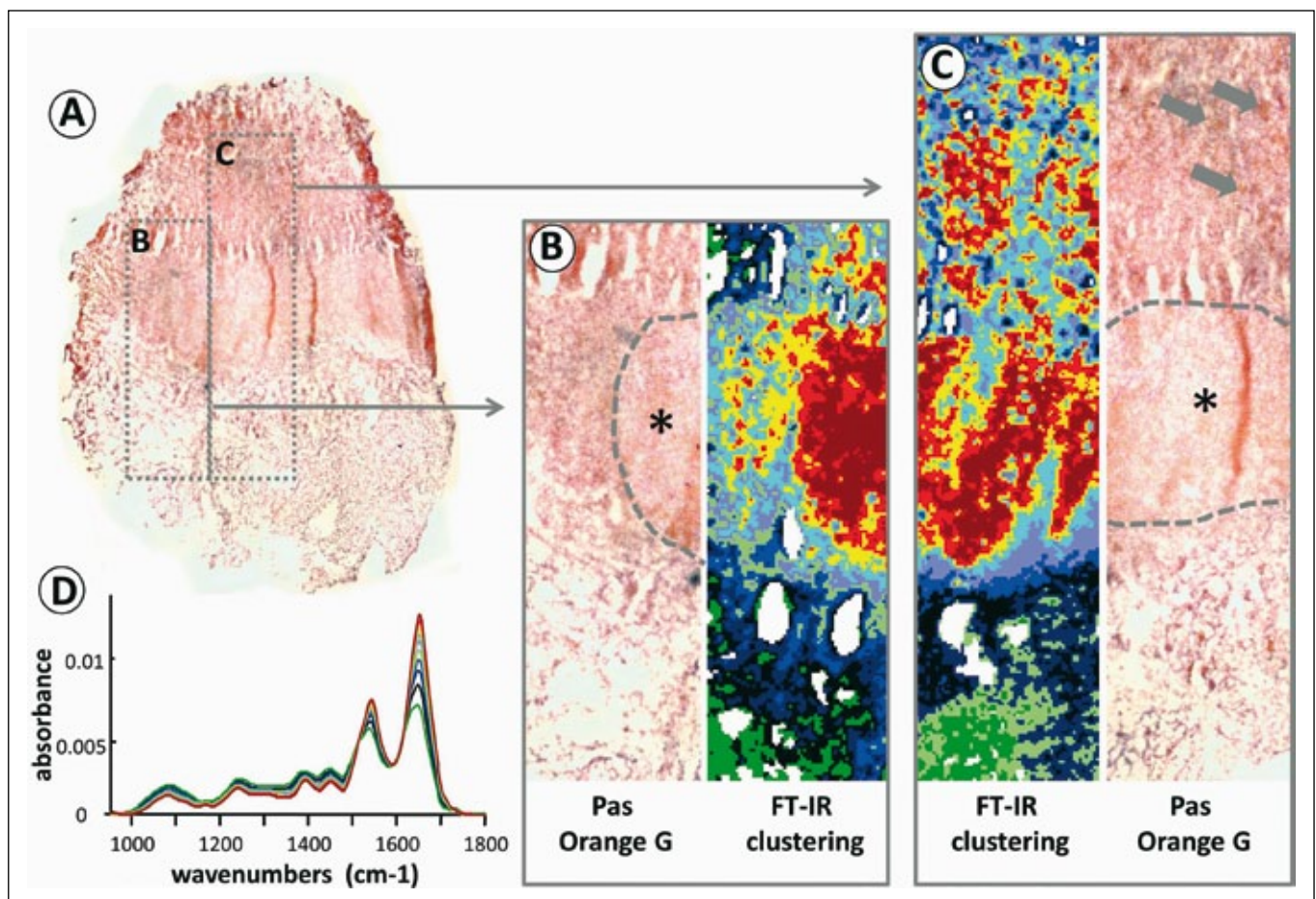


Figure 2. FT-IR imaging of human pituitary adenoma. **(a)** Overview of a cryosection of human pituitary adenoma stained using Pas-Orange-G and illustrating the production of human growth hormone (HGH). **(b, c)** High magnification of the areas indicated in **(a)** and corresponding FT-IR spectroscopic images obtained by cluster analysis. Large areas with increased HGH production are indicated by asterisks, local spots of hormone production are marked by arrows. **(d)** Representative spectra corresponding to area **(b)**.

FT-IR spectroscopy has been intensively used to investigate brain tumours. Primary brain tumours as well as brain metastases of peripheral tumours can be localized and discerned from normal brain tissue with high specificity [21–23]. In case of brain metastases, the type of primary tumour can be identified [24]. The main components that allow differentiation of normal and tumour tissues and tumour-grading are the tissue lipid content and changes related to nucleic acids [25]. Additionally, collagen content and distribution of collagen subtypes are altered in brain neoplasms and can be analyzed by FT-IR spectroscopy [26].

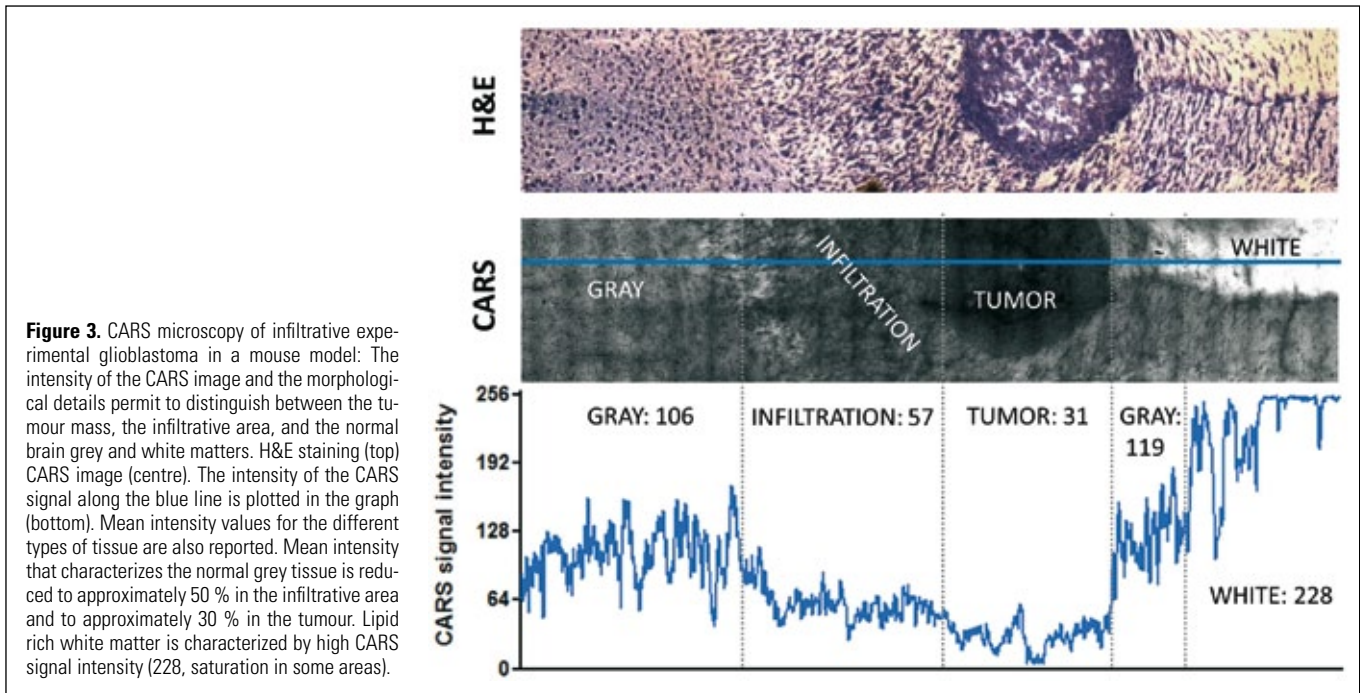
It was also possible to gain diagnostically relevant information, eg, the glioma grade, expression of hormones, or tumour vascularization can be assessed [27–29]. Figure 2 shows a tissue section of a human pituitary adenoma. Not only the tumour itself was identified but also the information about the pathologically and clinically highly relevant increased hormone production could be extracted from the spectral information using chemometrical analysis [28]. Cluster analysis sorts all spectra according to similarities and was used to build colour-coded maps of the tumourous tissue. Here, red pixels can be related to human growth hormone- (HGH-) producing tissue areas. In contrast, HGH-negative tissue areas are recognized in the blue clusters (Figure 2b,c). For cervical cancer, it has already been shown that infrared spectroscopy is able to make predictions at the molecular level [30].

ATR FT-IR

Attenuated total reflection FT-IR (ATR FT-IR) is a variant of infrared spectroscopy and offers the advantage of measuring non-transparent samples, eg, bulk tissue. This technique requires tight contact between the sample of interest and the core of the ATR device, the ATR crystal. Infrared radiation propagates in the crystal, generating an evanescent wave that penetrates a few micrometres of the sample. Spectral changes in the backscattered light are used to obtain information about the sample's biochemical properties. This is especially interesting for direct analysis of biopsy tissue [31]. Moreover, ATR FT-IR spectroscopy can be performed using a fibre optic probe [32] and can be implemented in an endoscopic setup. Therefore, it holds great potential for future clinical application and *in situ* diagnosis of malignant glioma. First results using optical ATR FT-IR spectroscopy for the analysis of native human brain tumour biopsies indicate the feasibility of this approach. It was possible to obtain high-quality spectra within minutes after tissue removal and to extract spectral differences in bands related to extracellular matrix components among different tumour types [31].

Raman Spectroscopy

Raman spectroscopy is based on the inelastic scattering of light and uses continuous wave lasers in the near-infrared for



excitation. In the Raman spectra, vibrational bands are better separated than in FT-IR spectra, therefore the spectra comprise a higher degree of information. Raman investigation of tissue permits to discriminate healthy from tumour and necrotic tissues in rat brain tissue samples [33] and was used to study brain functions in living mice and rats [34]. Brain injury caused by traumatic insults related to caspase-3-activated apoptosis can also be detected by Raman spectroscopy [35]. It has been demonstrated that Raman mapping can identify brain tumours in the living animal [36]. *Ex vivo* studies on human brain tumour samples have proven the ability of the technique to discern normal and tumorous tissues of adults [37, 38] and children [39]. Raman microspectroscopy of primary brain tumours can provide diagnostic information on the malignancy grade and cell density [40].

It has to be emphasized that the technique can be applied on native, non-dried tissue because the spectral contribution of water does not interfere with relevant bands of biological tissue as it does in FT-IR spectroscopy. Thus, artefacts due to drying and crystallization can be avoided [41] and, more importantly, this property makes Raman spectroscopy suitable for *in situ* diagnosis. First trials using fibre optic probes for *ex vivo* Raman spectroscopy of fresh human tumour samples proved the potential of the technique for grading of astrocytoma [42]. Current research focuses on the application of Raman spectroscopy to perform optical biopsies for tumour recognition [40], not only for brain tumours but also for other neoplasms eg, breast, skin, cervical, gastrointestinal, oral, and lung cancers [43].

Technical advances in the development and miniaturizing of Raman fibre probes may allow short acquisition times of approximately 10 s in concert with high-quality spectra acquisition [44, 45]. For gastric cancer diagnosis, Raman endoscopy has already been performed in a clinical context (> 300 patients) and provided diagnostic information [46].

Different techniques aim at enhancing Raman signal intensity in order to reduce acquisition time. Surface-enhanced Raman scattering (SERS) exploits the electrochemical interaction of molecules adsorbed by nanostructures. Applying the sample of interest onto a suitable surface enhances the Raman signal by as much as approximately 10^{10} . This technique is applicable for the analysis of chemical substances or single cells, but not for large tissue samples. Additionally, nanoparticles and compounds that exhibit strong SERS signals were employed as alternatives to fluorescent or colorimetric markers, and used for the detection and research of cancer and other diseases, eg, to visualize the distribution of known markers detected by classical immunohistochemistry [47]. In this context, spectroscopy is not used to reproduce tissue properties but to detect and reproduce the distribution of experimentally introduced compounds in a sample.

Resonance Raman spectroscopy exploits the amplification of the Raman signal that takes place when the energy of the exciting laser beam approaches the optical band gap of a tissue constituent, selected by appropriate tuning of the excitation wavelength. The Raman signal intensity is increased around 1000-fold and the resulting Raman spectrum is dominated by the bands of the resonance-enhanced molecule. Therefore, the main advantage over conventional Raman spectroscopy is mainly the ability to detect specific compounds at very low molecular concentrations, such as flavins, NADH, collagens, elastin, carotenoid, and the heme proteins [48].

The intrinsic characteristics of vibrational spectroscopic techniques, eg, being label-free and non-invasive, make them attractive for biomedical applications. However, these technologies generally require long exposure times and their lateral resolution is rather limited. Even if they offer great potential for detailed objective tissue classification in neuropathology (where time is not an issue) and are promising for *in situ* diagnosis of single suspect spots (lateral resolution/multiple acquisition).

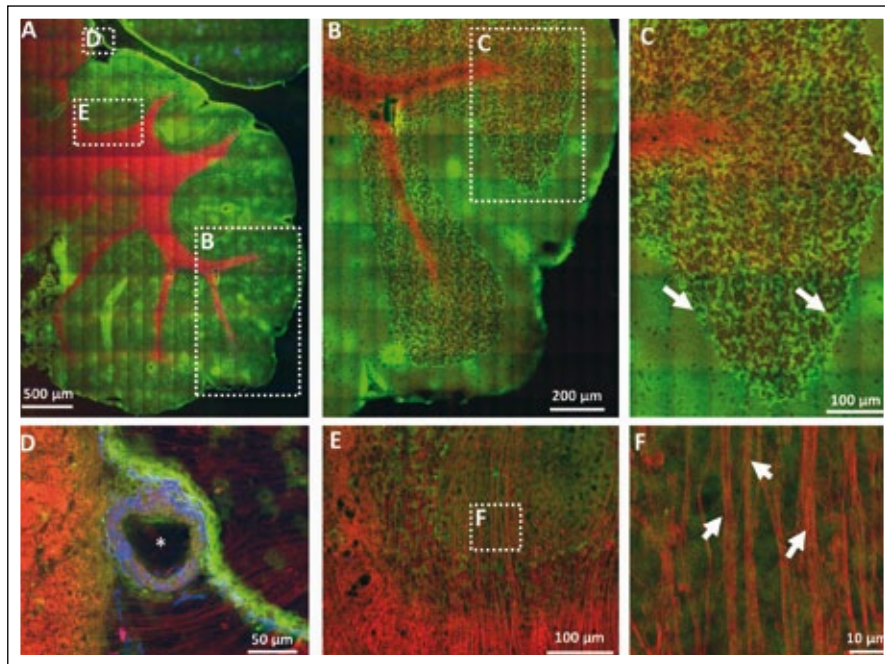


Figure 4. Multimodal NLO imaging of a mouse cerebellum (red: CARS, green: TPEF, blue: SHG). **(a)** Overview of the mouse cerebellum. **(b)** Magnification of the area indicated in **(a)**. **(c)** Magnification of the area indicated in **(b)**. Arrows indicate Purkinje cells characterized by punctuate fluorescence in the somata. **(d)** Magnification of the area indicated in **(a)**, a large blood vessel is shown (*). **(e)** Magnification of the area indicated in **(a)**, nerve fibres are indicated by intense CARS signal. **(f)** Magnification of the area indicated in **(e)**. Single axons can be discerned (arrows).

sitions are not required), their usefulness as an intraoperative imaging technology is limited.

■ Imaging Technologies

CARS Imaging

Nonlinear imaging techniques based on chemical contrast, such as coherent anti-Stokes Raman scattering (CARS), can overcome these limitations. CARS is a non-linear variant of Raman spectroscopy [49, 50]. It is based on resonant excitation of a single Raman band by using ultra-short tunable lasers and enables rapid acquisition of images. CARS images recorded at 2850 cm^{-1} mainly probe the spectral contributions of lipids [51, 52], which are important diagnostic markers of brain tumours [37], and enable visualization of tumour boundaries [51, 53]. In the CARS image, the tumour appears as a dark area surrounded by normal nervous tissue. This allows detection of primary brain tumours (gliomas), secondary brain tumours (metastasis) and of the tumour border with cellular resolution [54]. CARS imaging also permits to detect the infiltration of a tumour into normal, preserved brain areas. Figure 3 shows the CARS image and the corresponding H&E staining of a glioblastoma experimentally induced in a mouse brain. In the CARS image, the tumour can be recognized by its low CARS signal (dark region) and size and location matched the histological findings. The infiltrative zone was also characterized by a lower CARS signal intensity than in normal grey matter. CARS signal values along the blue line are shown in the graph and reveal the potential of CARS imaging for the development of an objective methodology for glioma analyses and characterization. Other tissue components may also be addressed by multiplex CARS and allow to address tumour-specific signals [55].

For possible intraoperative application, it is of ultimate importance to remember that CARS is a purely optical technique that requires no labelling but visualizes intrinsic tissue properties. So far, no phototoxic effects of CARS imaging have been shown to develop at the settings required for *in vivo* application and diagnosis [56].

Multimodal Nonlinear Optical Microscopy

CARS offers high-resolution imaging at video rate and can be combined with other nonlinear optical (NLO) microscopic methods on a single platform, allowing to retrieve a large amount of morphological and biochemical information from unstained, native tissue [53].

For multimodal NLO microscopy, the CARS signal (usually biochemical imaging of $-\text{CH}_2$ functional groups, ie, mainly lipids) can be acquired with other nonlinear processes that are simultaneously excited by the pulsed laser

sources used to induce the CARS signal. Acquisition of endogenous TPEF provides a nonspecific signal that contributes to the visualization of tissue structure and morphology. In some cases, endogenous fluorescence can be directly correlated with a certain cell type like inflammatory microglia/macrophages [57] or subcellular structures, eg, mitochondria, or elastin fibrils. Sources of endogenous TPEF within nervous tissue are, among others, NADPH, FAD, lipofuscin, or Schiff's bases. Endogenous fluorescence has already been used to discern the structure of normal brain and glioblastoma [58]. Acquisition of SHG allows the selective visualization of fibrillar collagen, which constitutes an important marker for malignant glioma [26] and indicates the presence of fibrous structures or connective tissue, as well as of tissue vascularisation due to adventitial collagen.

Figure 4 shows multimodal NLO images of a mouse brain cerebellum at different magnifications to illustrate the technology's potential. Detailed information about tissue structures and morphology can be obtained without the application of any labels or dyes on unstained, native brain tissue. CARS imaging (red) permits to visualize myelin-rich fibre tracts, allowing to access overall cerebral layering (low resolution, Figure 4a), fibre alignment (Figure 4e), as well as single axons (high magnification, arrows in Figure 4f). Endogenous fluorescence of the tissue (TPEF, green) also contributes to the visualization of the layering of the cerebellum (Figure 4b). Cell nuclei are lacking fluorescent signals and appear as dark spots. In contrast, Purkinje cells of the cerebellum exhibit a marked, punctuated fluorescence pattern in cytosol (Figure 4c, arrows). Large blood vessels can be recognized by second harmonic generation signal (SHG, blue) caused by collagen fibres of the vessel wall (Figure 4d).

Multimodal NLO microscopy, integrating CARS, TPEF, and SHG, was used for pathological assessment of human brain tumours on tissue sections and provided exhaustive imaging of the tumour morphology [54]. In the case of glioblastoma, it was possible to detect the border of the tumour by analyzing CARS signal intensity as shown for the orthotopic mouse model (Figures 5a,b). Additionally, numerous small blood vessels inside the tumour mass were identified by means of the SHG signal. Finally, in the case of neuroma (Figure 5c), it was possible to detect profound differences between tumour and normal nerve tissue: in the tumour, CARS and TPEF revealed the morphology of the tissue showing that the cells were loosely arranged in an interwoven pattern and SHG imaging visualized the extracellular matrix alterations typical of the tumour. These NLO images illustrate that multimodal biochemical imaging technologies can provide detailed information about tissue components and structures and permit to detect pathological tissue alterations. Automatized analyses allow to access diagnostically relevant tissue parameters like nuclear density and size in NLO images [59].

There are already commercially available solutions to investigate SHG and TPEF for the diagnosis of skin cancer (Dermainspect). Small, portable platforms integrating CARS, TPEF, and SHG imaging [60] as well as multiphoton endoscopes [61] have been developed and represent the first step towards bedside application of these new technologies.

Perspectives

At the present developmental status, CARS and multimodal imaging as well as vibrational spectroscopy can be employed in histopathology to gain additional information about brain tumour micromorphology and structure. Also the value of these label-free noninvasive methods for research needs to be recognized by the biomedical research community that usually applies labels or dyes to achieve visualization of tissue composition or of specific structures. Further research will then succeed in finding diagnostic and predictive markers using vibrational spectroscopy that is (compared to immunohistochemistry or molecular biology) cheap and fast.

The usefulness of biochemical imaging methods for neurosurgery has been widely demonstrated in proof-of-principle experiments with small sample sizes. Experiments have been performed *in vivo* in animal models [34, 36] and first studies have been performed on native *ex vivo* human brain tumour biopsies [31, 58]. Retrospective analyses indicate vibrational spectroscopy as a useful intraoperative tool for tumour border definition and detection of high-grade tumour residues [62]. To transfer these techniques into a clinical environment a large

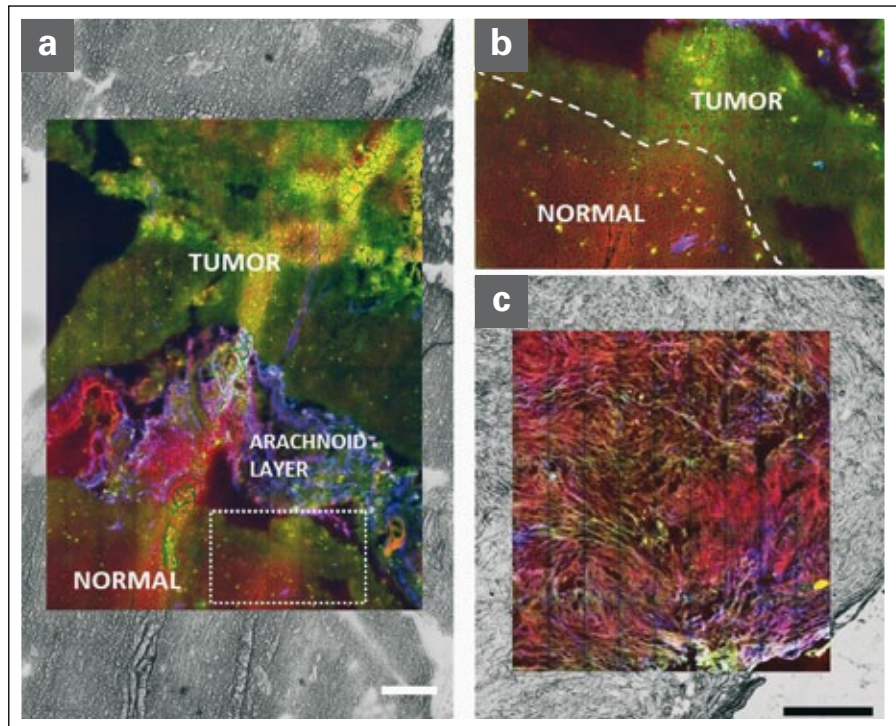


Figure 5. Multimodal NLO imaging of human tumours (red: CARS, green: TPEF, blue: SHG), overlaid with the bright field images of the unstained sample. The technique allows to retrieve detailed morphochemical information about tissue structure and properties on unstained samples. **(a)** Cryosection of human glioblastoma (scale bar: 200 μ m). **(b)** High magnification of the border between tumour and normal tissue as indicated in **(a)**. **(c)** Cryosection of human neuroma (scale bar: 0.5 mm).

set of glioma tumour samples is needed to match spectroscopic and clinical data of an extended set of patients.

In addition, technical engineering needs to improve miniaturisation for, eg, implementation in endoscopes, to improve scanning rates for usable imaging speed, and to verify the bioinformatics algorithms for data processing to extract and visualise relevant information in an adequate form to the neurosurgeon or neuropathologist. For routine intraoperative application, the surgeon needs an easy-to-handle device that delivers biochemical information about tissue status to be integrated in the established datasets, thereby facilitating and improving glioma resection. The compatibility with existing systems like surgical microscopes and endoscopes as well as with neuronavigation is essential for implementation in clinical routine.

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Conflict of Interest

The authors state that no conflict of interest exists.

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Awake Craniotomy in Glioma Surgery

Rachel Grossman, Zvi Ram

Abstract: Awake craniotomy for resection of gliomas aims to balance tumour removal with preservation of function. In this article, the authors provide a comprehensive review that summarises the most recent available data regarding vari-

ous aspects of awake craniotomy including the rationale for using this technique, the potential benefit, and the associated complications in the modern neurosurgical era. **Eur Assoc NeuroOncol Mag 2014; 4 (1): 27–33.**

Key words: awake craniotomy, malignant glioma, tumour resection, cortical mapping

■ Introduction

Maximal resection of malignant gliomas has been shown to be a favourable prognostic factor for survival. Maximal resection should be achieved, however, with preservation of the patient's neurological functions. Awake craniotomy with intra-operative mapping and monitoring of various neurological functions is a technology used by neurosurgeons to achieve that goal when tumours are located within or adjacent to functional brain regions. In this review, we discuss the rationale for maximal resection as a goal and describe the techniques used during awake craniotomy for intra-operative mapping and monitoring, including the pitfalls and complications associated with the technique.

■ Benefit of Maximal Tumour Resection

The value of maximal resection of malignant gliomas is controversial. There is consensus regarding the need to obtain pathological diagnosis and to reduce mass effect when present. However, there are no clear data supporting maximal surgical resection and enhanced overall survival, progression-free survival, or improved quality of life. 43 retrospective studies have been reported over the last 2 decades and examined the association between the extent of resection of low- and high-grade gliomas and survival. In 32 studies for high-grade gliomas (HGG), only 5 used volumetric MRI analysis. Three of the studies showed survival benefits of 2–8 months [1–3], and one study showed a trend toward enhanced survival, although it did not reach statistical significance [4]. Eleven studies assessed the correlation between extent of resection in low-grade glioma (LGG) and survival, with only 3 using volumetric MRI analysis [5–15]. All studies showed increased overall survival with maximal resection; in one, increased extent of resection resulted in a reduced incidence of malignant transformation [13].

■ Potential Benefits from Awake Craniotomy

Awake craniotomy with intra-operative mapping and monitoring has been reported to be associated with better neuro-

logical outcome, more extensive tumour resection, and shorter length of stay (LOS) in hospital [16–19]. Patients operated using awake craniotomy are not exposed to possible complications associated with general anaesthesia [20]. In fact, some data even advocate performing awake craniotomy as an outpatient procedure when patients are discharged after 6 hours of observation and a control post-operative CT scan [20]. In a series of 100 patients undergoing resection of supra-tentorial LGG without functional mapping, functional neurological outcome was compared to 122 patients with LGG who underwent tumour resection with intra-operative cortico-sub-cortical direct electrical stimulation [21]. The authors reported that in the non-mapped group 17 % experienced a new permanent neurological deficit compared to 6.5 % in the mapped group ($p < 0.019$). Moreover, postoperative MRI showed that GTR was achieved in only 6 % in the non-mapped group compared to 25.4 % of GTR in the mapped group ($p < 0.001$). Importantly, survival was directly correlated to the extent of resection [21]. In another retrospective analysis of patients with glioma who underwent surgical resection, 20 patients who underwent awake craniotomy were compared to 19 patients who underwent surgery under general anaesthesia. Awake craniotomy was associated with a shorter LOS in hospital [18].

■ Indications

Traditional indications for awake craniotomy with intra-operative mapping and monitoring include preservation of motor and language functions. Despite advances in functional imaging such as fMRI, diffuse tensor imaging (DTI), as well as intra-operative neuronavigation techniques, our ability to rely on these modalities to identify primary language sites preoperatively is still limited. Thus, awake surgery with intra-operative cortical stimulation-induced language dysfunction to identify functional language centres remains the gold standard for identification of these essential regions.

■ History of Awake Craniotomy

Awake craniotomy with intra-operative mapping was first used by Penfield [22] in the context of epilepsy surgery. Intra-operative cortical and subcortical mapping of eloquent areas has been used in brain tumour surgery during the last 2 decades. This technique provides the surgeon with real-time localization of functional regions in the brain and allows preservation of these regions by resulting in maximal and safe tumour resection.

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■ Description of the Technique

Preoperative Evaluation

Preoperative evaluation of patients considered candidates for awake-craniotomy with intra-operative mapping and monitoring at our centre includes formal speech evaluation that consists of naming, visual or auditory verb generation, and speech comprehension, which is usually carried out up to 2 days before surgery (baseline) and repeated intra-operatively. A comprehensive neuropsychological evaluation is performed and includes a short interview with the patient and a family member preceded by several cognitive tests such as WAIS III – Similarities and Digit Span, Rey Auditory Verbal Learning Test (RVLT), Rey-Osterrieth Complex Figure Test (RFLT), naming, verbal fluency (semantic and phonetic), WMS III – Special Span, and Stroop Test or MoCA Test [23]. The patient's emotional state and ability to cooperate during awake surgery is usually assessed by questionnaires, such as the Beck Depression Inventory, the State-Trait Anxiety Inventory (for quantifying anxiety), Barratt's Impulsivity Scale (BIS-11), and the Sensitivity to Reward and Punishment Questionnaire (SPSRQ) [24]. Patients also meet with a social worker and a member of the monitoring team for detailed explanation of the hospital course, including preoperative preparation, the nature of the surgical procedure, and the postoperative course. Minimal doses of sedatives and anxiolytic drugs are generally administered on the morning of the operation. Anticonvulsant blood levels are confirmed as being within the desired therapeutic level one day before surgery.

Intraoperative Management

Most patients at our centre receive intravenous midazolam (1–2 mg) and fentanyl (50–100 mcg) upon arrival at the operating room. Each patient receives nerve blocks with local anaesthetics and according to the location of the planned pinning and incision site, ie, supra-orbital, temporal, or occipital. Standard anaesthesia monitoring is accompanied by invasive blood pressure monitoring. Spontaneous ventilation is monitored by capnography. Because urinary catheters are not routinely inserted (found to be particularly disturbing in men), we try to avoid the administration of mannitol or over-hydration. All patients receive oxygen (3 l/min) through a nasal cannula during surgery. Light sedation is achieved intra-operatively with continuous administration of remifentanyl when needed

(Figure 1). In certain situations, such as when sedation with remifentanyl does not seem to be adequate, propofol is supplemented under careful supervision. Patients who exhibit a low baseline speech level do not receive propofol or remifentanyl at all. All sedatives or analgesics are discontinued briefly after pinning of the skull in order to carry out a second neurocognitive evaluation after the patient's head has been immobilized and before incising the skin. Patients who experience pain from dural manipulation are injected with lidocaine 1 % between the dural leaves. Evaluation of performance in all tasks is assessed by comparing accuracy and speed of response to the preoperative levels. Mild sedation and pain control medication are provided after the resection is completed until the skin incision is closed.

Mapping

Traditionally, mapping is performed with 50-Hz stimulation, however, other strategies are available. At our centre, we use direct cortical 50-Hz bipolar stimulation for cortical mapping of speech and motor functions (Ojemann Cortical Stimulator, Radionics, Burlington, MA) [25]. The cortical surface is stimulated in 2-mA increment intensities, from a baseline of 4 mA to a maximum 10 mA, or until functional response is elicited. Effects of stimulation on behaviour and performance (eg, speech arrest, anomia, hesitation, error in finger tapping, any motor responses) are noted, and the anatomical and radiological locations as well as the intensity applied are recorded.

Monitoring

The tasks used for the purpose of monitoring during surgery vary according to the proximity of the patient's lesion to cortical areas of language functions or according to the patients' specific functional MRI mapping of language functions (as measured by blood oxygen level-dependent activation). Language is checked by free speech after cortical stimulation and throughout the resection itself by sub-cortical stimulation of adjacent tracts (ie, superior longitudinal fascicle, arcuate fasciculus etc). Motor functions are evaluated according to the tumour location by asking the patient to perform various movements (eg, clenching a fist, flexing a foot) as well as by the ability of the patient to plan and initiate movements in cases of lesions in proximity to the supplementary motor area. Motor-evoked potentials and corresponding muscle responses are monitored in most patients.



Figure 1. Intra-operative photographs of the surgical team during awake craniotomy.

■ Neuroanaesthesia in Awake Craniotomy

Neuroanaesthesia in awake craniotomy is important for keeping the patient cooperative during the mapping phase as well as for decreasing the physical and psychological stress associated with this procedure. There are several anaesthesia protocols in use. Intermittent general anaesthesia with controlled ventilation for asleep-awake-asleep (AAA) has been described. A laryngeal mask or endotracheal tube is inserted before the beginning of the operation and taken out before mapping is started. At the end of the mapping phase the patient is re-anaesthetized and ventilated through the laryngeal mask or endotracheal tube. In a prospective single-centre study, 140 patients were operated for tumour resection under AAA. They were fully awake for a mean of 98 minutes, discomfort was reported in 17.8 %, with one case of aspiration, and no mortality. AAA has the advantage of sparing the unpleasant phase of craniotomy and hence does not have a time limitation during non-eloquent tumour resection or during the closure phase. However, the main disadvantage is the risk of coughing and aspiration [26]. In our experience, patients are operated under monitored anaesthesia care with no general anaesthesia. Patients are spontaneously breathing throughout the entire procedure. Sedation is achieved with propofol and remifentanyl during the insertion of the head holder and craniotomy itself. Once the mapping phase is complete sedation is re-applied. The main complications associated with general anaesthesia are minimized by this approach. Minimal sedation does not usually lead to airway obstruction, hypoxia, or hypercapnea.

■ Surgical Technique: The Evolution of Craniotomy Size

Intra-operative mapping was first used in the context of epilepsy surgery. In this procedure, large craniotomies with wide cortical exposure are used to identify cortical regions responsible for language and motor function (ie “positive sites”). A different approach has evolved for tumour surgery. Tumour resection is performed through smaller, tailored craniotomies with small cortical exposure of the tumour region. Tumour resection is directed through non-critical cortical regions (absence of stimulation-induced language responses, and sometimes without localization of positive functional sites [27]). This strategy is more time-efficient [28]. However, despite minimizing the risk of causing neurological deficits, these complications may still occur [29].

■ Intra-Operative Monitoring

Language

Significant inter-individual variability in language site organisation exists. This may be due to an anatomical variation mass effect carried by the tumour or even brain reorganisation due to brain plasticity. Speech arrest may be produced during awake craniotomy far beyond the classical Broca’s region. Importantly, it is crucial to differentiate between dysarthria and speech arrest. Speech arrest is recognized by ceasing fluent function (ie, number counting) without simultaneous involuntary motor response in the muscles affecting speech. Since functional brain tissue may be located inside tumours such as malignant gliomas, it is not always safe to presume that the tu-

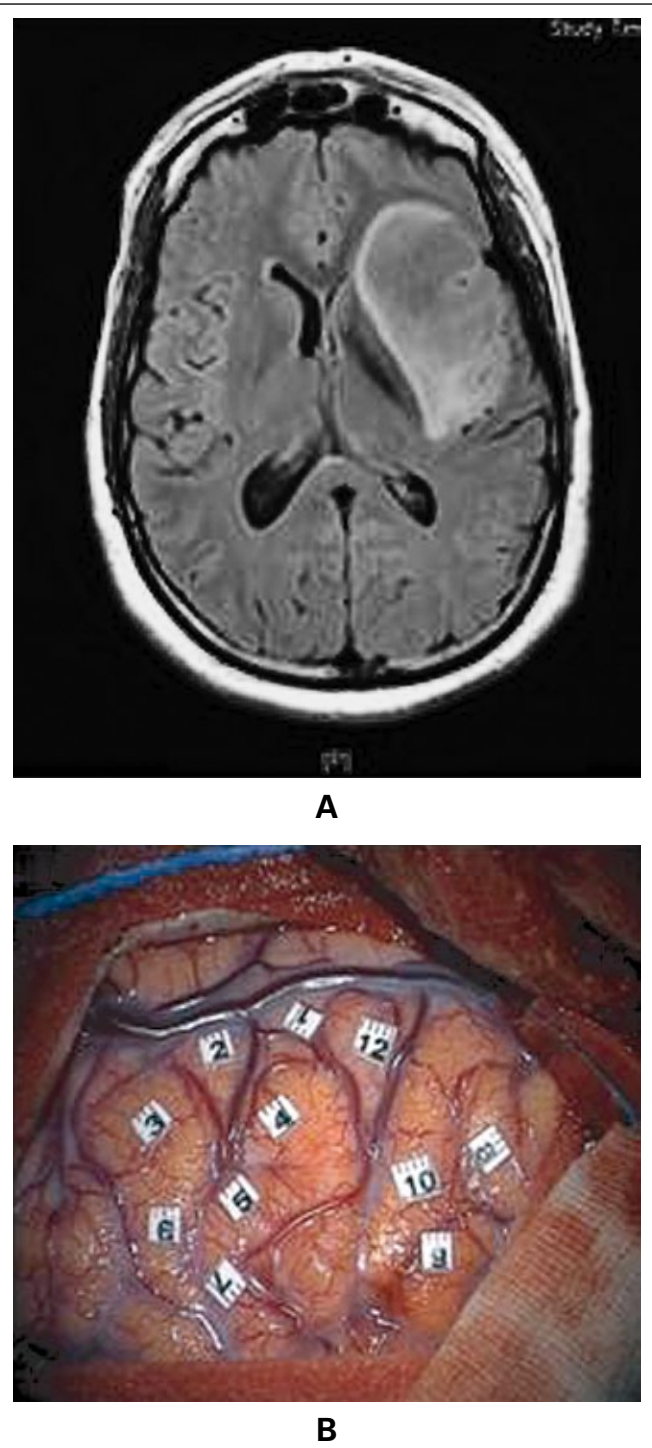
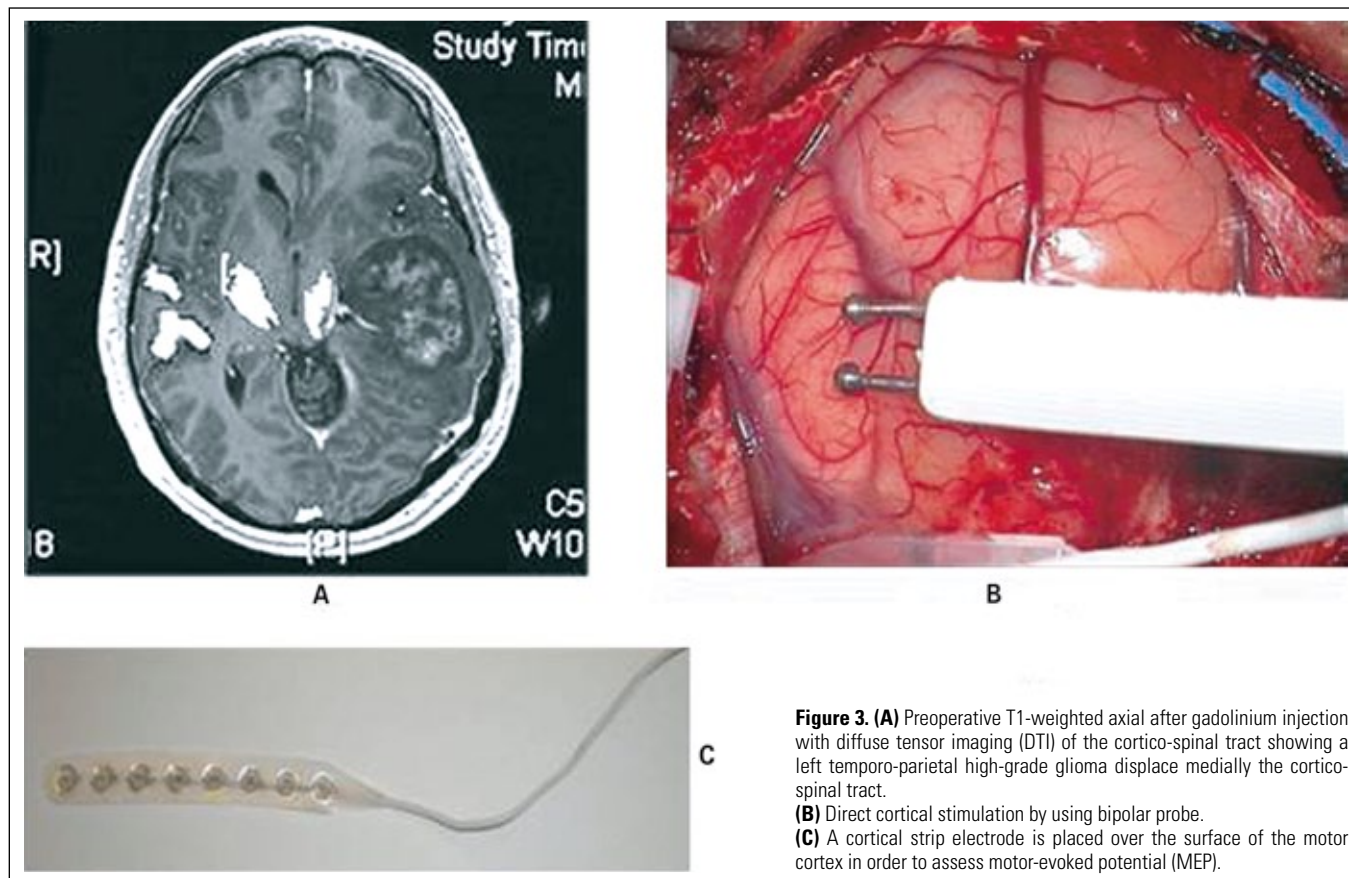


Figure 2. (A) Pre-operative axial fluid-attenuated inversion recovery- (FLAIR-) weighted MRI showing a WHO grade-II insular glioma in a 40-year-old woman presenting with episodes of seizures. This patient has no neurological deficit. (B) Intra-operative cortical mapping of language during awake surgery.

mour’s interior can be resected without functional deterioration. This is the overall rationale for operating lesions located adjacent to presumed speech centres by awake surgery (Figure 2). Brain tumour resection with intra-operative language mapping was performed in 250 patients with gliomas [27]. Tailored craniotomies with limited cortical exposure resulted in only few points of localization of language sites (“positive sites”). Still, 6 months later, only 1.6 % had a persistent language deficit [27]. Interestingly, in a case report of a patient with anaplastic astrocytoma located in Broca’s area, only subtotal awake resection was achieved. This patient underwent



implantation of a subdural grid over his Broca centre within the residual tumour. Continuous high-frequency cortical electrical stimulation (cHFCS) was applied with stimulus intensity that caused mild speech disturbances. After 25 days of stimulation the author reported displacement of speech function and the patient underwent second surgery during which

the tumour could be completely removed with no deficit. The authors concluded that this phenomenon was evidence for induction of topographic plasticity by using cHFCS, which allowed more extensive tumour resection [30].

Motor Function

Motor function is a complex process involving several frontal regions including primary motor cortex, secondary motor areas such as the supplementary motor area (SMA), pre-motor area, and cortico-spinal tracts (CST). Electrical stimulation of the primary motor cortex leads to motor response (ie, movement). Electrical stimulation of certain secondary motor areas such as the pre-motor area and caudal SMA may lead to cessation of voluntary movement. This was first described by Penfield and Jasper [31], and defined later by Lüders et al as “negative motor areas” [32].

Direct cortical stimulation is conducted by bipolar probe with 2 tips at 6–10 mm distance with frequencies of 50–60 Hz. Initial stimulation intensity is usually 2 mA and gradually increased in 2-mA increments up to 10 mA. Somatosensory evoked potential (SSEP) is used for phase reversal and identification of the central sulcus. Electromyography (EMG) is used to record motor response in muscles. In addition to continuously assessing the integrity of the motor pathways, motor-evoked potential (MEP) monitoring is conducted by placing a strip electrode (4–8 contacts) over the surface of the pre-central gyrus. MEPs are recorded by needle electrodes inserted into the contra-lateral muscles (same as use for the EMG). During tumour resection sub-cortical stimulation is applied, usually with monopolar probe to identify the proximity to the motor pathways [33] (Figure 3).

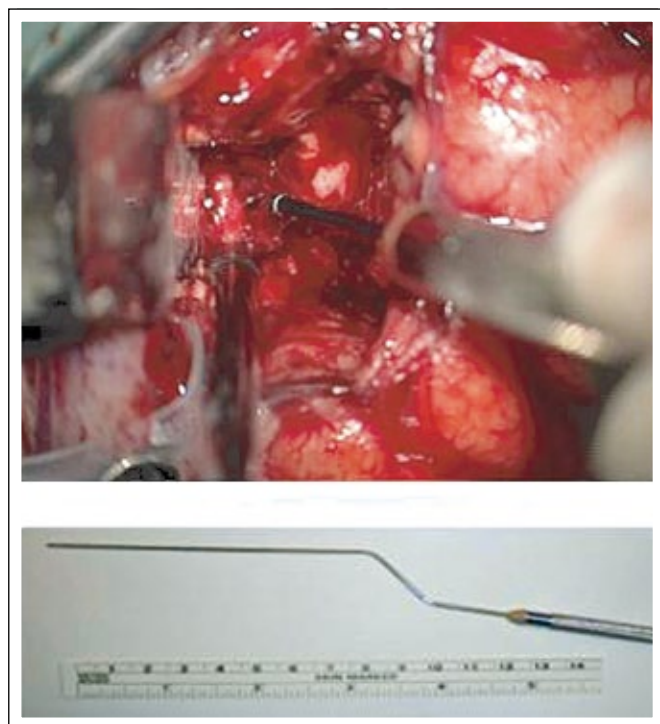


Figure 4. Sub-cortical stimulation of the cortico-spinal tracts by means of a subcortical monopolar electrode.

In a retrospective analysis in 55 patients with tumours located within or adjacent to the cortico-spinal tract (CST), direct cortical-stimulated motor-evoked potentials and sub-cortical stimulated motor-evoked potentials were assessed and the current intensity used to get motor response was correlated to the distance from CSTs. A linear correlation was found between the distance from CSTs and the threshold of sub-cortical stimulation producing a motor response (0.97 mA for every 1 mm brain tissue distance from CSTs) [33] (Figure 4). Importantly, direct cortical and sub-cortical stimulation can be achieved in awake manner or under general anaesthesia (while avoiding the use of halogenated inhalational and muscle paralysis agents). Our experience shows no significant difference between those methods with regard to immediate post-operative motor status or extent of resection [33].

■ Outcome of Patients Undergoing Awake Craniotomy

The usefulness of awake surgery with intra-operative cortical and sub-cortical mapping has not been assessed in randomized trials. There is conflicting data based on retrospective studies comparing the outcome of patients with brain tumours located adjacent to the eloquent cortex who were operated under general sedation versus local anaesthesia (awake craniotomy). The most extensive work recently published in the literature is a meta-analysis of 90 reports with 8091 patients who underwent resective surgery for malignant supra-tentorial gliomas with and without intra-operative stimulating mapping. Severe late post-operative neurological deficits were observed in 3.4 % of the patients who underwent intra-operative mapping, and in 8.2 % of patients who were operated without intra-operative mapping [34]. GTR was achieved in 75 % and 58 % of these groups, respectively. The authors have concluded that glioma resection using intra-operative stimulation mapping is associated with less late and severe neurological deficits and more extensive resection and should thus be widely used as a standard of care in glioma surgeries [34].

■ Patient Satisfaction

Awake craniotomy has been shown to be a safe procedure from a medical point of view. However, several studies investigated patient perception and satisfaction with the procedure [20, 35, 36]. Specifically, their levels of anxiety, expectations, and satisfaction were assessed. It appears that awake craniotomy is well-tolerated by those patients who understand the benefits of the procedure and believe that it may improve their post-operative outcome. Patient recollections of the procedure vary. Some patients have no recollection at all while others vividly remember the sound of the drill or the pressure on the head during the insertion of the Mayfield head holder. Interestingly, most patient recalls are auditory memories (suction, drilling, voices of the surgical team). Half of them do not remember the cortical mapping part despite being fully cooperative during the procedure. They remember talking with the surgical team, being asked to perform tasks such as to move their extremities, and answering questions. Overall, despite experiencing minimal pain and discomfort during certain parts of the operation (placement and removal of cranial fixation, skin incision), it appears that most patients

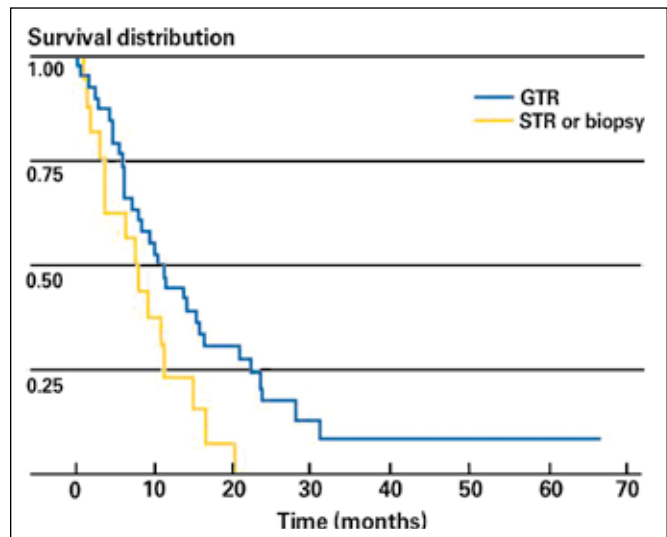


Figure 5. Kaplan-Meier curve of survival for elderly patients (65 years) with high-grade gliomas (HGG) who underwent awake-craniotomy gross tumour resection (GTR) compared to subtotal resection (STR) or biopsy. Median survival was significantly longer for patients with HGG who underwent GTR compared to those who underwent STR or biopsy (10.8 vs 7.8 months, respectively; $p = 0.039$) [39].

tolerate awake craniotomy well and show an overall high level of satisfaction [20, 37].

■ Awake Craniotomy in Special Populations

Awake Craniotomy in the Elderly Population

Despite data linking awake surgical resection of supra-tentorial tumours with maximum extent of resection and overall better outcome, older patients with malignant brain tumours are rarely offered such aggressive treatment. We compared the outcome of 90 elderly patients (> 65 years old) who underwent awake craniotomy to 334 patients < 65 years old. Specifically, the 2 groups were compared for surgical outcome parameters such as postoperative complication, mortality, LOS, and overall survival [38]. There was no higher rate of mortality or post-operative complications in the elderly group of patients, except increased LOS. On average, elderly patients tend to stay 2 days longer in hospital compared to their younger counterparts. Interestingly, a subset of elderly patients with HGG enjoyed a significant survival benefit after awake GTR of their tumours (Figure 5). Taken all of these data together, it appears that awake maximal surgical resection of brain tumours in the elderly population with good pre-operative functional status is feasible, safe, is not associated with increased peri-operative morbidity or mortality, and may increase their survival. Unfortunately, there is no additional data investigating this topic.

Awake Craniotomy in the Paediatric Population

Awake craniotomy has been used in the paediatric population [40–42]. Specific challenges concern cooperation, understanding, and anxiety. Several reports have been published so far including a case of a 9-year-old girl with NF1 who underwent successful awake craniotomy with intra-operative language mapping for resection of a left temporo-parietal GBM [40]. Another report described 2 patients who were 16 years old when diagnosed with intra-axial brain tumours located adjacent to their speech brain regions. They both underwent

intra-operative language mapping and resection of their tumours. Overall, it seems that awake craniotomy is feasible and safe also in the paediatric population, but is undoubtedly challenging in younger children, especially the part of brain mapping. Additional neurophysiological strategies are warranted.

■ Complications

In addition to the complications associated with craniotomy under general anaesthesia such as post-operative infection or post-operative bleeding, there are several complications that are uniquely associated with awake craniotomy.

Awake Craniotomy Failure

Awake craniotomy may need to be converted in certain situations into general anaesthesia surgery. There are several causes that may lead to failure of the procedure and unsuccessful stimulation and mapping. In a review of 424 patients undergoing awake craniotomy, 27 were considered as an awake craniotomy failure [43]. The most common reason was lack of intra-operative communication with the patients ($n = 18$; 4.2 %), mostly due to pre-operative dysphasia and phenytoin treatment. As one can expect, their outcome compared to those who underwent successful awake surgery was worse in several parameters: their overall complication rate was significantly increased with a higher incidence of postoperative dysphasia, the rate of GTR was significantly lower, and their length of stay was significantly longer with an average of an additional 3 days in hospital.

Intra-Operative Seizures during Awake Craniotomy

Seizures are commonly presenting symptoms in patients with brain tumours occurring in 30–50 % prior to their diagnosis. Intra-operative seizures during awake craniotomy may have a negative impact on the surgical course and in certain cases may require conversion into general anaesthesia. The postictal period may negatively affect patient cooperation, especially during electrical cortical and sub-cortical mappings. This may cause reduced tumour resection and a higher rate of post-operative neurological deficits. In a review of 477 patients with brain tumours who underwent awake craniotomy, the incidence of intra-operative seizures was 12.6 % ($n = 60$). Eleven patients (2.3 %) were considered as a failure while awake craniotomy was converted into general anaesthesia surgery. Interestingly, these failed patients tended to be younger with a history of pre-operative seizures. In general, their outcome was worse with a significantly higher rate of short-term motor deficit, and they tended to stay longer in hospital [39]. In a series of 511 patients who underwent tumour resection with intra-operative brain mapping, 25 (4.9 %) patients experienced intra-operative seizures. Two patients required intubation and induction of general anaesthesia [44]. It is well-known that tumour histology and location may predict seizure occurrence. The incidence of seizures is higher in patients with LGG and is more common when the tumour is located in the frontal and temporal lobes. In a series of 137 patients with gliomas, the occurrence of intra-operative seizures during awake craniotomy was 21.1 %. A significant correlation was found between intra-operative seizures and tumour location. Specifically, patients with tumours located in the supplementary motor area

had the highest incidence of intra-operative seizures (73.3 %) regardless of their seizure history.

■ Intra-Operative Monitoring of Cognition

Intra-operative mapping of non-language or motor function has received less attention. Many patients suffer from post-operative visual and cognitive deficits that negatively affect their quality of life. Thus, there is a need to expand the indications for awake craniotomies for preservation of their functions.

Mapping of SMA

The SMA, part of the frontal lobe, is responsible for the initiation and planning of movements. Unilateral SMA lesion may lead to motor and language deficits. This usually gradually improves within a few weeks (SMA syndrome). Rosenberg et al [45] investigated SMA activity in 26 patients with tumours located in this region. All patients underwent awake tumour resection with motor and language monitoring. The motor paradigm consisted of finger tapping that was validated before by fMRI studies to activate the SMA [46]. In this task, the patient had to plan sequences of finger movements. Language was assessed by verb generation tests and free speech. Task dysfunction during direct cortical stimulation was associated with critical involvement of the SMA in this task. Stronger activation of the lesioned SMA was seen in patients without direct cortical stimulation-induced dysfunction. The authors suggested that this phenomenon was the result of higher functional sites that were able to compensate for the disruption caused by electrical stimulation.

Mapping of Optic Radiation

Post-operative visual deficits have received little attention in the past and have been considered an acceptable postoperative neurological deficit although they may have negative consequences on daily activity. Epilepsy surgeons traditionally used anatomical criteria to preserve optic pathways during mesio-temporal surgeries. However, it appears that there is much inter-individual variability in the optic pathways, especially the part located posterior to the lateral geniculate body [47, 48]. A series of 14 patients with grade-I, -II, and -III gliomas who underwent awake tumour resection with intra-operative mapping of the optic radiation using sub-cortical electrical stimulation has been recently reported. These patients had tumours involving the optic radiation with none of them having had a pre-operative visual field deficit. During surgical tumour resection, direct sub-cortical electrical stimulation was repeatedly performed until the optic radiation was identified by transient visual disturbances. All patients experienced visual disturbances during mapping and tumour resection was stopped at this point. Only one patient experienced permanent post-operative hemianopsia [49].

Future Directions

Individually Tailored Mapping

Mapping of non-language, non-motor, essential functions such as spatial perception and memory has received less attention and is considered highly experimental. Individually tailored mapping is desired not only in the sense of portraying functional organisation at the individual patient level, but also in terms of selecting the functions to be mapped. Ideal-

ly, the selection process should rely not only on tumour location and clinical symptoms but also on each patient's particular occupational needs and habits (eg, perception of tempo and pitch for musicians, sense of space for pilots or civil engineers) to improve surgical outcome for this particular patient. Indeed, several clinical reports have recently published the use of awake mapping for various tasks with good clinical outcome. There is one case report describing intra-operative mapping of calculation in a school teacher operated for resection of a left parietal tumour involving the angular gyrus [50]. She was neurologically intact before surgery and during mapping performed some mild serial arithmetical subtraction errors. She underwent GTR of the tumour and left with a mild post-operative deficit for arithmetical subtraction. In another report, 4 amateur singers, pre-operatively intact, with brain tumours who underwent awake surgery using a singing task during direct cortical stimulation, demonstrated clear distinction between speech and singing in the Broca region [51]. We have recently related pre-operative fMRI activation in the supplementary motor area and the functional deficit aroused during intra-operative direct cortical stimulation [45].

■ Conflict of Interest

None.

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Subacute Spinal Cord Compression Due to an Intramedullary Spinal Teratoma in a 53-Year-Old Female Patient: A Case Report

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Introduction

Spinal teratomas are a rare disease and constitute for 0.1–0.5 % of all spinal tumours [1, 2]. They can be divided into extra- and intradural and extra- and intramedullary teratomas according to their location [1, 2–5].

A teratoma is an encapsulated tumour with tissue or organ components resembling all 3 germ layers. Due to their capsule they are usually benign (mature) tumours. A malignant immature form can be found in male children. In adults, teratomas are more frequent in women.

We report the clinical and radiological characteristics of a 53-year-old woman with subacute progressive paraparesis. After spinal surgery, an intramedullary teratoma was histologically diagnosed.

Case Report

A 53-year-old woman complained about increasing lumbago over 10 days. On admission, she reported a bilateral L4 radicular pain syndrome, more prominently to the right. Neurological examination showed neither weakness of the lower extremities nor any sensory deficits. Lasegue's sign was negative on both sides. Medical history revealed the diagnosis of Guillain Barré syndrome in 2007 without any neurological sequelae.

Lumbar MRI, which was performed after admission, showed an intraspinal intramedullary tumour at level Th11/Th12, with an axial diameter of 3 cm and a sagittal diameter of 1.5 cm, associated with a myelopathy up to Th10. T₂-weighted images showed slightly inhomogeneous hyperintense signals. In the T₂ STIR sequences, the tumour appeared homogeneously hypointense. Administration of contrast medium exhibited a homogeneously strong enhancement. Altogether, the tumour had a lipoma-like appearance in accordance with its homogeneous tissue (Figure 1).

After admission, oral and intravenous analgesic treatments were administered. Initially, the patient responded well to this treatment of pain. Five days after admission, severe subacute progression of spinal signs and symptoms took place. Clinical neurological examination revealed a sensomotor transversal deficit at level Th11 including a maximal deficit of muscle strength of the left lower limb of 1/5 and the right lower limb of 3/5 (British Medical Research Council [BMRC] scale).

After paraparesis onset, intravenous dexamethasone treatment was established and neurosurgical intervention was decided on.



Figure 1. MRI of the thoracic and lumbar spine at admission, showing the teratoma at levels Th11/Th12.

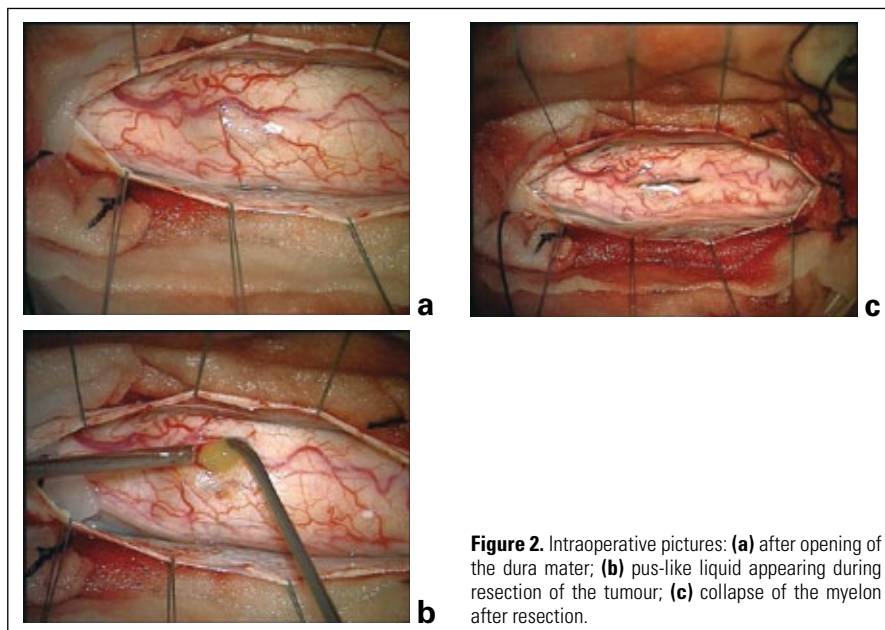


Figure 2. Intraoperative pictures: (a) after opening of the dura mater; (b) pus-like liquid appearing during resection of the tumour; (c) collapse of the myelon after resection.

With the guidance of intraoperative neurophysiological monitoring and ultrasound, standard laminectomy at Th11 and subsequent myelotomy were performed (Figure 2). The conus was enlarged and after myelotomy a muddy, milky, yellow and, to some extent, brownish fluid was drained (Figure 2b). The entire cyst was washed out and a biopsy specimen of the cyst wall was taken. Other types of tissue such as hair, bones, or skin were not found. We stopped further manipulation to avoid postoperative neurological deficit and closed the field.

Histopathological findings revealed a mature intramedullary teratoma consisting of mostly adipose tissue and also parts of dermal tissue.

After surgery, the profound weakness as well as the sensory deficit of the lower limbs subsequently improved. Neurological examination 10 days after operation revealed almost normal muscle function. Only a discrete weakness of the left foot extension remained.

After 3 weeks of neurorehabilitation, routine MRI follow-up investigation showed regular postoperative results (Figure 3). After 6 months, only a light hypaesthesia in dermatoma L4 on the left limb was found during clinical examination.

Discussion

Benign intramedullary teratomas in adults causing spinal cord compression with painful subacute paraparesis are rare. This case study reports clinical and neuroradiological features as well as treatment and outcome.

According to a review by Poeze et al [6], 31 out of 83 teratomas were of the intramedullary type, and 52 cases were intradurally extramedullary or extradurally located. The occurrence of spinal teratomas not associated with dysraphism is rare and more common in infants and adolescents than in adults.

Several authors have reported that the thoracolumbar region is most commonly affected, particularly in the area of the conus medullaris [6–9].

Most of the cases reported in the literature presented with weakness of the lower extremities, sensory changes, and reflex abnormalities, related to the site of the tumour [6]. Spinal pain syndrome was only found in 1/3 of the patients. However, a subacutely developing painful paraparesis in a patient with intramedullary teratoma has not been reported in the literature so far.

Our patient also had neurological signs and symptoms in accordance with the location of the teratoma. However, most patients described in the literature were younger and did not present with such a clinically fast progressive spinal cord compression.

Due to the rapid progression 5 days after admission we suspected haemorrhagic transformation of the tumour. This could not be verified intraoperatively. The cystic components were



Figure 3. MRI of the thoracic and lumbar spine after partial resection.

to some extent brownish but not in the sense of a recent intracystic/intratumoural haemorrhage.

MRI is regarded as the gold standard diagnostic technique for the detection of the location of the teratoma and the degree of spinal-cord involvement. Concerning radiological features, usually mixed high- and low-intensity signals reflect the cystic and solid components of a teratoma [10]. In our patient's MRI, typical characteristics of a lipoma were mimicking a teratoma, which in retrospect represented the more lipomatous tissue of the tumour.

Complete resection of the tumour is the treatment of choice in spinal teratoma. Due to its intramedullary location only a biopsy of the cyst/tumour wall could be performed in our patient. In general, total resection of intramedullary spinal teratomas seems difficult due to potential neurological complications and frequent adhesions to the surrounding neural parenchyma [7–9, 11].

Conclusion

This is the case of an intramedullary spinal teratoma in a 53-year-old female patient who presented clinically with a rapidly progressive spinal cord compression. The radiological features of the lesion were mimicking a lipoma. Despite fast progression of clinical signs and symptoms surgical decompression and cyst evacuation of the teratoma led to a good clinical outcome. Due to a possible tumour recurrence we recommend annual clinical and radiological follow-up investigations.

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Dysphagia in Glioblastoma

Marike Kamphuis, Hanneke Zwinkels

■ Introduction

Patients with a malignant brain tumour often experience signs of dysphagia. These so-called swallowing problems may cause aspiration, pneumonia, dehydration, malnutrition, asphyxia, and difficulty in medication intake. Occurrence of dysphagia in glioma patients is described with a variable frequency, ranging from 26–85 % [1, 2]. Oberndorfer et al [3] report an increased incidence of swallowing problems in the 10 weeks before death. Patients are often not aware of the fact that they suffer from dysphagia [4], and therapeutic interventions are seldom applied [3].

At the Medical Centre Haaglanden, speech and language therapists are consulted for patients with brain tumours who are admitted to the hospital and who have difficulties speaking and swallowing. A retrospective investigation of the files of 56 patients showed that dysphagia was diagnosed in 55 % of patients. Brain tumours located in the right hemisphere (n = 15) were all associated with dysphagia, those located in the left hemisphere (n = 39) were responsible for dysphagia in 30 % of patients. Two patients, of whom one had a bi-frontal tumour and one a pontine glioma, both suffered from dysphagia.

During the end-of-life phase, interventions for dysphagia are often difficult. In general, swallowing problems occur mostly while drinking. It is advised to use thickening powder (Nutrilis, Resource Thicken Up) in order to prevent patients from choking. Patients often experience the use of thickening powder as less tasteful, but it might take away the risk of developing aspiration pneumonia. Finding the right balance between the amount of thickening powder and tasteful food is a challenge. Slightly thicker liquids like chocolate milk, butter milk, and fruit juice can often be used without thickening powder. Pudding, yoghurts, soft curd cheese, and apple sauce are often easier to swallow. Mixed consistencies such as vermicelli-soup and yoghurt with cereals are more difficult to swallow, because the oral musculature has to adapt to different consistencies with a different speed of movement. Medication intake is more simple when crunched and presented with thickened liquids, but this is not always allowed. Adjustment of oral medication into powder, liquid, or injection could offer a solution.

■ Case Study: Dysphagia in a Glioblastoma Patient

A 64-year-old male patient was diagnosed with a glioblastoma multiforme in his right frontal-temporal lobe for which he underwent resection and concomitant radiotherapy and temozolomide.

The tumour was diagnosed after 4 months of changed senses of smell, less sensibility and tingling of the left side of his body. Concomitant treatment with radiotherapy and chemotherapy was discontinued because of a deterioration of his clinical condition. One year after the first signs and symptoms the patient died.

First contact between the speech therapist and the patient took place shortly after tumour resection. Post-operatively, the patient suffered from left-sided hemiparalysis. Speaking was tiresome but when articulating consciously, he could make himself understood. There was no problem with swallowing but tongue movements were asymmetric. Anticipating future problems, the speech therapist gave advice and education on swallowing.

Two months later the patient was admitted to the hospital because of neurological deterioration during chemoradiation. Besides an asymmetric tongue, there was a paralysis of the facial nerve and weakened articulation, altogether resulting in choking on drinks. To avoid choking, we advised to use thickening powder. Apart from thickening powder a normal diet was advised. During hospitalisation, swallowing was observed several times, leading to adjustments of the amount of thickening powder, resulting in safer and more tasteful drinking. It was also noticed that the patient was taking huge gulps. When advised to take smaller sips, the patient complained less frequently of choking.

Before the patient was discharged from hospital to go home, his spouse informed the speech therapist that she was afraid her husband would choke and regretted the lack of support from the medical team. Supportive educational intervention by giving information to the spouse was provided which she greatly appreciated.

■ Conditions for Safe Swallowing

The patient has to be in a stable upright position, preferably in a chair at the table or otherwise with good support in bed.

Take time for consuming food and drinks, offer/take small sips or bites at a slow pace. Wait until the amount has been swallowed before offering the next sip or bite.

Help the patient concentrate on swallowing and do not speak during eating or drinking. This could decrease the risk of aspiration by inhaling during swallowing.

Glioma patients often have delayed oral reflexes that cause choking. Use a spout cup appropriately. Do not tilt the head backwards too far. This causes a rapid movement of the liquid

to the throat and as a result requires a faster swallowing reflex. A straw is often positioned at the back of the mouth which causes delayed innervation of the swallowing reflex. It is better to position the straw on the lips.

■ Choking

Choking is expressed by coughing and gasping, a veiled voice, tears, or flushing. It is important to have the patient cough independently, as long as necessary to remove the choked substance from the trachea. It is advised not to hit or tap on the back of the patient because this would interrupt the natural rhythm and power of cough. Wait until the patient has stopped coughing before offering the next sip. If necessary, the Heimlich manoeuvre can be applied or the throat can be sucked out.

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Greater Collaboration Leads to Greater Knowledge and Hope

The First World Summit of Brain Tumour Patient Advocates Takes Place in Lafayette, California, Just Prior to SNO/WFNO 2013

Kathy Oliver

The world of brain tumour patient advocacy had never seen anything like it.

From Argentina to Zimbabwe and Australia to the USA, 64 participants from 20 countries arrived in Lafayette, California, on November 18, 2013, to make history at a 2-day conference (November 19 and 20) aimed at increasing knowledge and collaborative efforts for the benefit of brain tumour patients and caregivers around the globe¹.

Nearly 2 years in the planning, the “First World Summit of Brain Tumour Patient Advocates” – organised by the International Brain Tumour Alliance (IBTA)² – provided a unique opportunity to meet like-minded people from different countries; exchange ideas on best practice, challenges, and advocacy experiences, and build new relationships between organisations and individuals in the advocacy arena.

Attendance at the Summit was by invitation. The countries represented were: Argentina, Australia, Belgium, Canada, Denmark, India, Ireland, Italy, Japan, Lithuania, Netherlands, New Zealand, Norway, Singapore, Spain, Sweden, Taiwan, United Kingdom, United States, and Zimbabwe.

■ Collaboration and Sharing

Just as the major international neuro-oncology and cancer scientific congresses strive to bring together global leaders in these fields so that they might interact with each other, learn from each other, and cooperate long-term, so the IBTA's Summit assembled the movers and shakers, the Board Chairs and CEOs of leading brain tumour advocacy organisations around the world.

The main theme of the Summit was “collaboration” and “sharing”. The 48-hour conference was professionally facilitated by a leading patient advocacy expert. The Summit was collegially designed so that nearly all of the participants either presented in a plenary session, spoke as part of a panel, ran a workshop or marketplace, acted as rapporteurs, or assisted in a practical way to ensure a smooth meeting.



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Participants from around the world gather in Lafayette, California, to attend the IBTA's First World Summit of Brain Tumour Patient Advocates.

The IBTA's international team of 11 advisors provided advice, help, and support throughout the process of devising and holding the Summit.

■ Something for Everyone

The Summit featured different types of sessions so everyone had a chance to participate in a way in which they felt comfortable and confident about actively contributing to the programme.

A highlight of the Summit was the excellent introductory overview of current brain tumour treatments presented by neurosurgeon and past president of the Society for Neuro-Oncology (SNO), Dr Susan Chang.

Over the 2 days of the Summit, the 9 plenaries included a “Setting the Scene” session which introduced some of the issues faced by brain tumour patient advocates in their work such as: grappling with health technology assessment (HTA), accessing innovative therapies, caring for the caregiver, support and information provision, personalised medicine – the hype and the hope, challenges of paediatric brain tumours, and help for brain tumour patients in the developing countries.

Also featured in the plenaries were lectures on brain tumour statistics, country-specific challenges and solutions for brain tumour care and support, exploring some of the unmet needs of the brain tumour community, and sharing best practice.

¹ For the biographies of participants at the First World Summit of Brain Tumour Patient Advocates, see <http://www.theibta.org/BiosAndPhotos.pdf>

² International Brain Tumour Alliance – www.theibta.org

An innovative “Marketplace” session kept everyone literally on their toes. This part of the programme involved Summit participants moving in small groups between 6 different locations in the Summit hotel where facilitators conducted 30-minute “taster” sessions on various topics including fundraising in an era of austerity, how doctors can improve their communication with brain tumour patients, working with volunteers and the art of succession planning/sustainability of patient organisations, working with industry, social media and the IT revolution, and working with print and broadcast media (including public service broadcasts).

■ Workshops Ask Searching Questions

Five parallel workshops were also held at the Summit. These 75-minute sessions, led by Summit participants, addressed the following questions:

1. How can clinical trials be better organised for brain tumour patients?
2. What do brain tumour patients want from pharmaceutical companies in order for companies to be more ‘patient-centric’?
3. What do brain tumour patients want from the organisations that represent them?
4. What are the relationships like between brain tumour patient groups, cancer control organisations, and the neurological disease community?
5. How can we adequately support people with a low-grade or meningioma brain tumour?

Summaries were produced from all the plenaries, Marketplace sessions, and workshops with the aim of publishing a post-Summit report which will be released early in 2014.

■ A Remarkable, Historic Event

This historic Summit was remarkable in many ways. Collectively, the brain tumour advocacy leaders at the Summit represented over 500 years of experience in helping patients, their caregivers, and families on their journeys.

The large majority of brain tumour patient advocates at the Summit had never before met, yet the spirit of cooperation, determination, and teamwork permeated the 2 days of the Summit as if everyone had known each other for many years.

A deep camaraderie quickly developed among the Summit participants who all came from different cultural, socio-economic, geographic, and religious backgrounds. United for 2 days, these once-strangers from the 4 corners of the world seemed to find in each other a deep friendship and empathy, forged from their common desire to significantly improve the journey for brain tumour patients and their caregivers.

Indeed, 42 of the 64 participants were either brain tumour patients themselves, current brain tumour caregivers, or former brain tumour caregivers.

One participant at the Summit commented, “*It was great to be able to compare notes on running a brain tumour patient or-*

ganisation. But even more meaningful was to share our personal brain tumour journeys with others who really understood, from the depths of their souls, what it was like to see someone you love diagnosed with a brain tumour. This unique bond that we share is deep and powerful.”

Other participants at the Summit who had become involved in the brain tumour world, but not because of a personal connection to the disease, nevertheless brought to their roles in their organisations – and the Summit – a truly impressive commitment to improving the situation for our community through their determined and dedicated work.

■ Advocacy Challenges from Around the World

The Summit included not only advocates from developed countries, but also those from the less developed areas of the world such as Zimbabwe. The founders of the Zimbabwe Brain Tumour Association described how they have to work in a highly chaotic environment, greatly influenced by their country’s problems of rocketing inflation, political and economic instability, fuel and food shortages, and the collapse of the healthcare system.

Advocacy colleagues representing The Brain Tumour Foundation of India spoke compellingly of the challenges in their country: for example, the stigma of a brain tumour diagnosis, the numerous linguistic dialects, the very low level of public awareness about this disease, the limited surgical suites and imaging modalities, the overall health focus on communicable diseases, and the cultural bias toward superstition which affects the efficacy of healthcare.

A Lithuanian brain tumour colleague described the absence of information and organizational support as major barriers in his country.

Summit participants heard from colleagues in the USA, Europe, Japan, Australia, and Canada that even in the richest and most powerful countries on earth, patients can still be lost in a maze of uneven and inequitable care. In addition, brain tumour therapies do not easily fit into the regulatory systems and they certainly do not come cheap, causing challenges with their reimbursement and equitable access in countries with national health systems.

Representatives from Argentina, Singapore, New Zealand, and Taiwan described the situation for brain tumour patients and caregivers in their own countries and shared insights into what their particular challenges are.

An added value of holding the Summit in California in November was that participants, if they wished, could then attend the SNO/WFNO meeting in San Francisco after the Summit³. For some advocates, it was their first time at a high-level international scientific conference. SNO was extremely supportive

³ 4th Quadrennial Meeting of the World Federation of Neuro-Oncology in conjunction with the 18th Annual Meeting of the Society for Neuro-Oncology, see <http://www.soc-neuro-onc.org/2013-annual-meeting/>

of the Summit, demonstrating its commitment to the patient advocacy movement and its warm welcome of collaborative events of this nature.

■ Predicting the Future by Helping to Create It

The Summit examined, on many levels, what really matters to brain tumour patients and caregivers on their journeys. But it also looked at the road ahead.

One of the best ways to predict the future is to help create it. The Summit hopefully marked a turning point in the delivery of a better future for those whose lives are touched by this disease.

To achieve this, members of the international brain tumour advocacy community should push strongly for more support, more information, more research, and quality care for our patients. We should collaborate with others to help avoid duplication and waste of precious resources.

We should help, as much as we can, those brain tumour patients in the less developed countries of the world, too.

One Summit participant wrote after the meeting, *"Together our efforts for the brain tumour population will grow and not go unheard by government, healthcare workers, and our patients and families."*

Another participant added, *"It was an enlightening experience to listen to stories [from] across the globe. The challenges the brain tumour patient and caregiver face are truly universal. Everyone shared so many remarkable stories of faith, endurance, and hope. It was sad for all the loss, but amazing to hear how many people are making a difference for others ... [on] the brain tumour journey."*

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Congress Preview: ASNO Conference to Be Held in Istanbul in September 2014

Türker Kiliç

On behalf of the Organizing and Scientific Advisory Committees of the ASNO 2014 Conference, I would like to invite you to join us in Istanbul from September 11–14, 2014.

Every year, scientists and professionals in the general field of neuro-oncology in Asia come together for this high-profile scientific meeting. Featuring speakers and participants from all over the world, this conference acts as a platform that provides an ample opportunity for researchers, clinicians, and professionals not only to share information but also to establish a network essential for future collaborations.

The scientific programme will be innovative and of the highest quality, with keynote and invited speakers among whom will be Koichi Ichimura, Jeong Hoon Kim, Zhong-ping Chen, David Reardon, Martin Taphoorn, Necmettin Pamir, Kumar Somasundaram, Mark Rosenthal, Tai-Tong Wong, Santosh Kesar, Hugues Duffau, Charles Stiles, Michael Weller, Kyung Gi Cho, Ryo Nishikawa, Liang-fu Zhou, Yukitaka Ushio, Jun Yoshida, Rakesh Jalali, Masao Matsutani, Riccardo Soffietti, Ham-Min Tseng, Chae Yong Kim, Yong Kil Hong, and Hee-won Jung.

The special pre-meeting courses offered are “Multidisciplinary management of high-grade glioma course” (organised by

Susan Chang), “Skull base anatomy course” (organised by Albert Rhoton and William Couldwell), and the “Neuropathology course” (organised by Kaoru Kurisu and Ozlem Yapicier).

Based on the increasing number of participation over the past few years, we expect to host nearly 1000 attendees from around the world in Istanbul, a city known for its unique historical wealth and cultural attractions. As a bridge between the east and west, Istanbul will be the perfect backdrop for this international meeting. The cusp of summer and autumn, September is one of the best times in the year to visit Istanbul and enjoy thousand years-old churches, mosques, palaces, and the blue waters of the Bosphorus.

All of us on the ASNO committees look forward to seeing you in Istanbul for ASNO 2014.

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The EUROPEAN ASSOCIATION of NEUROONCOLOGY



11th MEETING

2014

October 9-12

Turin, Italy

Lingotto Convention & Exhibition Centre

Abstract Deadline: March 23, 2014

Early Registration: June 15, 2014

Please find further information on
www.eano.eu

Invitation to Turin

Dear Colleague, dear Friend,

The Italian Association of Neuro-Oncology (AINO) is honoured to host the 11th EANO 2014 meeting in Turin, and invites you to join this outstanding event for neuro-oncology in Europe and worldwide.

Over the last two decades there has been an explosive growth of knowledge and interest in neuro-oncology. Nowadays the proper management of neuro-oncological patients requires specific experience and training. The EANO is a multidisciplinary and multiprofessional society aiming to provide science, standards of care, education, and communication. In this regard, EANO 2014 will give the opportunity to share the most up-to-date advances in basic science, translational and clinical research. All fields of neuro-oncology will be covered, such as biology and pathology, imaging, surgery, radiotherapy, chemotherapy, targeted therapies, supportive/palliative care and quality of life.

Turin is an attractive city located in Northern Italy between the Alps and the Mediterranean sea. Turin was the first Italian capital between 1861 and 1865 and its historical importance is shown by many architectural masterpieces, like the Mole Antonelliana, which is the hallmark of the city and the Reggia di Venaria, a Royal Residency. Moreover, the Egyptian Museum is the second most important in the world.

From the beginning of the 20th century, Turin became a strong industrial centre. The Lingotto building is an example of the industrial transformation of the city. In the last years, Turin changed once again its attitude and mood, hosting the 2006 Winter Olympics Games and becoming a more international and welcoming city.

We look forward to welcoming you in Turin in 2014.

Roberta Rudà
Scientific Secretary
Organising Committee
EANO 2014
(on behalf of AINO)



Riccardo Soffietti
President, EANO
President, EANO 2014



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Congress Preview: International Symposium on Pediatric Neuro-Oncology (ISPNO)

Singapore, June 28 to July 2, 2014

Stewart Kellie

The International Symposium on Pediatric Neuro-Oncology (ISPNO) is the major biennial global meeting of the multi-disciplinary international community of professionals involved in the research, diagnosis, treatment, and rehabilitation of infants and children with brain tumours.

The 16th meeting will take place from June 28 to July 2, 2014, and will be held in conjunction with the 8th St. Jude-VIVA Forum in Pediatric Oncology in Singapore for the first time. Since the first ISPNO meeting in 1986, this biennial scientific conference has witnessed progress that was unimaginable in the 1980s. The pace of discovery and our knowledge of biology, treatment, and late effects of paediatric CNS tumours has increased dramatically but many challenges remain, particularly in translating these advances to clinical care in developing nations.

ISPNO is the forum of choice for researchers from North America and Europe to announce paediatric neuro-oncology research results. The meeting encompasses advances in molecular diagnostics and classification, the chemotherapy of tumours of the CNS in phase-III trials, results of trials of investigational new agents from phase-I and -II trials, and the latest in supportive care.

Professionals involved in the care of children and adolescents are encouraged to benefit from this valuable opportunity to

engage with experts, gain insights, and establish new collaborations from the enriching plenaries, poster programme, and more.

Throughout the entire symposium, attendees will engage in dialogue regarding new surgical treatments, innovative research, and advances in paediatric neuro-oncology in a dynamic and interactive forum designed to significantly expand the knowledge base of attendees and further enhance overall patient care worldwide.

Visit www.ispno2014.com for complete information, registration, accommodations, etc.

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National Societies: The Belgian Association of Neuro-Oncology

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The Belgian Association of Neuro-Oncology (BANO) was founded on May 26, 2005, in order to promote multidisciplinary treatment of brain tumours and research in neuro-oncology. At that time, more than 200 patients per year were diagnosed with glioblastoma in Belgium and the EORTC had just presented data showing the benefit of radio-chemotherapy with temozolomide for glioblastoma. This association aims to be a meeting point for all physicians involved in the treatment of brain tumours and also for other societies involved in neuro-oncology research. At present, BANO has 110 members coming from different specialities such as neurosurgery, radiation oncology, medical oncology, pathology, radiology, nuclear medicine, psychology, and nursing. This non-profit organisation is financed by membership fees and project-related grants.

This association has 2 missions: (1) to promote neuro-oncological research and (2) to improve the health care standards for the multidisciplinary treatment of brain tumours patients.

BANO has been involved in several clinical research projects. Initially, the association provided access to temozolomide for patients with relapsed grade-III gliomas. We have set up a randomised national clinical multicentre trial evaluating the potential benefit of prolonged adjuvant treatment with temozolomide for glioblastoma patients. 62 patients have been included and the final results will be available soon. Another project is under discussion with the EORTC regarding our participation in the intergroup study exploring the role of temozolomide in the post-operative setting of grade-III tumours harbouring a co-deletion of 1p and 19q.

To pursue our mission of permanent improvement of health care standards for brain tumour patients, every year we organise a national symposium. This gives us the opportunity to

invite top-class speakers who are all experts in their respective fields. Our last symposium (2013) focused on the current impact of genetic biomarkers in the diagnosis and treatment of primary brain tumours. These events usually attract around 100 physicians who are daily involved in the treatment of brain tumour patients. During this event, the Belgian Brain Tumour Support (BBTS) offers 2 prizes for young researchers in order to promote research. We have also built a website to provide physicians with all relevant information regarding the national symposium, guidelines, and contact details of physicians involved in neuro-oncology.

The association is also committed to defending the interests of brain tumour patients in various medical societies and on the level of the regulatory authorities in a growing number of occasions. Recently, BANO was involved in a project set up by the Ministry of Health to define criteria for hospitals to take care of rare cancers such as primary brain tumours under the guidance of the KCE (Kennis Centrum/Centre d'Expertise).

We thank all the physicians dedicated to neuro-oncology research and health care for their support of the association. In the future, BANO wishes to continue to contribute to all the work that is still needed to improve the survival and the quality of life of our beloved patients.

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L. Bello, W. Grisold, A. Hottinger, F. Lefranc, C. Marosi, K. Rössler, R. Soffietti, W. Wick

Interview with Dr Stephanie E Combs, Heidelberg University Hospital, about the Phase-II Trial Evaluating Carbon Ion Radiotherapy for the Treatment of Primary Glioblastoma (CLEOPATRA)

Ufuk Abacioglu

From the Department of Radiation Oncology, Neolife Medical Center, Istanbul, Turkey

Q: Dr Combs, what can you tell us about the ongoing phase-II trial on carbon ion radiotherapy in newly diagnosed glioblastoma? What are its background and objective?

A: Glioblastomas are highly treatment-resistant tumours, and an improvement of the therapeutic window is being intensively sought for in several disciplines and aspects. As far as radiation is concerned, they are known as radio-resistant lesions, therefore, methods of dose escalation have been studied with more or less success. Particle therapy is characterised by a distinct physical profile, with a sharp dose deposition within the Bragg peak and a steep dose fall-off thereafter. Thus, normal tissue can be spared effectively. Carbon ions are also associated with a higher relative biological effectiveness (RBE). In my group, we could show that this RBE lies between 3 and 5, depending on the cell line and endpoint, and several analyses have shown additive effects together with chemotherapeutic agents. Thus, the CLEOPATRA trial evaluates a carbon ion boost to the macroscopic tumour with the aim of exploiting the biological benefit of carbon ions.

Q: How is the trial designed? Which patients are eligible?

A: The trial is designed as a randomised phase-II trial, the primary endpoint is overall survival at 12 months. Main inclusion criteria include histologically confirmed primary glioblastoma after biopsy or partial resection and indication for combined chemoradiation with temozolomide.

Q: Previous dose escalation studies have been disappointing with regard to improving outcome and showed increased toxicity. What is the rationale behind this trial to change the dismal prognosis?

A: The aim is not only to exploit dose escalation but also the biological properties of the carbon ion beam. Several preclinical studies have shown a higher RBE and the physical properties of particle beams allow dose deposition precisely to the defined tumour while sparing surrounding tissue. Also, in contrast to older dose escalation trials, improved imaging for target volume delineation including modern MR sequences as well as PET imaging has been implemented. This is also likely to improve the therapeutic window.

Q: What are the dose and fractionation schedules in both arms?

A: In both arms, patients are treated with 50 Gy photon radiotherapy as chemoradiation with temozolomide to the T2-

hyperintense region including the necessary safety margin. Thereafter, randomisation is performed, in both arms particle therapy is applied. The standard arm includes 10 Gy E, 5 fractions of 2 Gy E up to a total dose of 60 Gy with protons, in the experimental arm 18 Gy E of carbon ions in 6 fractions are applied.

Q: What are the target volume definitions in the trial?

A: Gross Tumour Volume (GTV) is defined as the contrast-enhancing lesion on MRI as well as PET-positive regions. For the base plan, the clinical target volume (CTV) includes the GTV, the T2-hyperintense regions including a safety margin of 2–3 cm. The boost is defined with a boost CTV including the GTV plus a 5-mm safety margin. The PTV is added according to institutional guidelines, as the 50-Gy base plan can also be applied in external institutions and patients come to Heidelberg for boost treatment only.

Q: Are you planning to conduct any translational studies?

A: At our department, blood samples are being collected from all patients within a clinical protocol for subsequent translational projects. Moreover, molecular markers for glioblastoma will be correlated with outcome to identify potential subgroups of patients showing distinct responses and outcomes. However, these measures are not part of the inclusion criteria and immediate study protocol.

Q: How is response assessed in this trial? Do you use specific imaging modalities for response assessment and follow-up?

A: Of course, response criteria include, as outlined in the most recent RANO guidelines, clinical as well as imaging parameters. MRI is used as a regular follow-up and amino-acid-PET is scheduled for specific questions, ie, to differentiate pseudoprogression, radiation reaction, and tumour progression.

Q: How is accrual progressing and when can we expect final data?

A: We are currently still recruiting patients, aiming at a projected number of 150 patients. So, we hope to be able to report some data from the interim analysis when the patient and event numbers defined have been reached.

Q: Finally, can you comment on the very unique names chosen for your trials?

A: I myself always find it difficult to remember trials whose identification only consists of numbers, such as EORTC or RTOG trials. Therefore, I find it helpful to give each trial a special name. It makes it easy for our treating physicians, physicists, and study nurses to keep track on the trial and the patients in them. Also, I found it very positive when patients can remember the name of “their trial” and identify themselves as being members of that trial. Currently, CLEOPATRA has also some Disney sisters, CINDERELLA for recurrent gliomas and MARCIE for atypical meningiomas, but we are also recruiting into some trials with names based on Greek-mythology characters, such as PROMETHEUS, PANDORA, and PHOENIX.

Thank you!

Dr Stephanie Combs is currently vice chairman of the Department of Radiation Oncology in Heidelberg, Germany, and the study coordinator of the CLEOPATRA trial.

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Hotspots in Neuro-Oncology

Michael Weller

From the Department of Neurology, University Hospital Zurich, Switzerland

■ Rituximab Is Associated with Improved Survival for Aggressive B Cell CNS Lymphoma

Gregory G, et al. Neuro-Oncology 2013; 15: 1068–73.

Despite the absence of evidence for its activity, the CD20 antibody rituximab has been broadly introduced into the primary treatment of primary central nervous system lymphoma (PCNSL). In the August issue, Gregory et al from Australia sought to validate this approach by a retrospective study of 128 patients with PCNSL treated with or without rituximab. On univariate analysis, younger age, ECOG performance status 0 or 1, normal lactate dehydrogenase, diagnosis after 2004, and treatment with cytarabine and rituximab were predictive of favourable overall survival. On multivariate analysis, only age was an independent predictor of survival. The interpretation of these results is difficult. Given that treatment outcome is likely to improve due to many reasons over time and that multivariate analysis failed to show a role either for rituximab or for treatment after 2004, I doubt that this data can be used to justify the routine introduction of rituximab into standards of care for PCNSL. Prospective randomised trials are the only way to move this complex field forward.

■ Subsequent Brain Tumors in Patients with Autoimmune Disease

Hemminki K, et al. Neuro-Oncology 2013; 15: 1142–50.

Interrelations between various autoimmune diseases and the incidence and cause of various brain tumours have remained a controversial topic in the epidemiology of brain tumours. No firm conclusions have been derived from previous studies. For the present study, data were derived from Sweden. Data on 402,462 patients hospitalised for autoimmune diseases were studied, brain tumour risks and survival were analyzed and compared with those of the general population. Among 33 autoimmune diseases, none had an impact on the incidence of brain tumours; however, there was a negative impact on survival both in gliomas and in meningiomas. The reasons for this remain unclear but may include enhanced physical disability or under-treatment because of limitations of cancer treatment by the pre-existing autoimmune disorder. Thus, while there have been claims that gliomas may even be underrepresented in patients with allergies because of enhanced immune reactivity, no link between the evolution of brain tumours and autoimmune dysfunction was disclosed here.

■ Memantine for the Prevention of Cognitive Dysfunction in Patients Receiving Whole-Brain Radiotherapy: A Randomized, Double-Blind, Placebo-Controlled Trial

Brown PD, et al. Neuro-Oncology 2013; 15: 1429–37.

Whole-brain radiotherapy has been increasingly challenged in the treatment of various neuro-oncological conditions because of insufficient efficacy and its unfavourable, notably long-term safety profile. Efforts at improving or maintaining cognitive function in irradiated patients have so far not been successful. In the October issue, Brown et al, for the Radiation Therapy Oncology Group (RTOG), presented results of a randomised controlled trial which assessed the possible benefit of the candidate neuroprotective agent, memantine, in brain metastasis patients treated with whole-brain radiotherapy. Although the mode of action of memantine in other neurological conditions has not been fully clarified, antagonism at the N-methyl-D-aspartate type of the glutamate receptor is currently thought to mediate these effects. Although the trial failed to meet the primary endpoint, in part as a result of insufficient power, secondary endpoints and subgroup analysis indicated that memantine-treated patients had superior cognitive function over time. These interesting results provide support for further exploration of similar pharmacological approaches in patients with brain metastases for whom whole-brain radiotherapy seems to be indicated and indispensable. Various candidate drugs are in principle available and similar trials are eagerly awaited.

■ The Immune Cell Infiltrate Populating Meningiomas is Composed of Mature, Antigen-Experienced T and B Cells

Fang L, et al. Neuro-Oncology 2013; 15: 1479–90.

Meningioma is not commonly a tumour considered to trigger a significant immune response. Moreover, it has not been a target disease for the increasing number of immunotherapy approaches currently applied to other brain tumours, notably glioblastoma. In the November issue, Fang et al assess the immune cell infiltrate of a series of 28 meningiomas and observed significant numbers of B and T cells, in particular around vessels. In most tumours, flow cytometry of *ex vivo* harvested tumour tissue was used to further characterise T cell phenotypes. The tumours commonly contained antigen-ex-

perienced CD4+ and CD8+ memory/effector T cells, regulatory T cells, and T cells expressing PD-1 und Tim-3. These T cell populations were enriched compared with peripheral blood. These studies highlight that there are specific interactions even between meningiomas and the immune system and illustrate the possibility for future immunological approaches particularly in tumours where surgical and radio-oncological treatment strategies have failed.

■ Seizure Control Following Radiotherapy in Patients with Diffuse Gliomas: A Retrospective Study

Rudà R, et al. Neuro-Oncology 2013; 15: 1739–49.

Symptomatic epilepsy is a common presenting sign and associated with significant impairment of quality of life in patients with low-grade gliomas. Since imaging endpoints have remained controversial in these patients, measures of clinical benefit would be helpful in defining a role for any treatment in

patients with low-grade gliomas. In the December issue, Rudà et al reported on the effect on seizure control of radiotherapy in patients with diffuse gliomas. In a retrospective study of 43 patients with low-grade gliomas, there was a significant reduction of seizure frequency in 31 of 43 patients (73 %) at 3 months after radiotherapy. Seizure freedom was achieved in 32 % of all patients at 12 months. Effects on seizures were not necessarily reflected by effects visible on MR imaging. These interesting study results suggest that the impact of therapeutic measures for low-grade gliomas on seizure frequency should be incorporated as an endpoint in future clinical trials.

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SNO News: Recap of the 4th Quadrennial Meeting of the World Federation of Neuro-Oncology

Nicholas Butowski

The 4th Quadrennial Meeting of the World Federation of Neuro-Oncology was held in conjunction with the 18th Annual Scientific Meeting and Education Day of the Society for Neuro-Oncology from November 21–24, 2013, in San Francisco, CA. The meeting enjoyed record attendance of close to 2100 registrants from 46 different countries.

We congratulate meeting chair Dr Mitchel Berger for composing a comprehensive programme which highlighted cutting-edge laboratory and clinical research. Special thanks are also extended to the scientific planning committee which was comprised of members from each of the 3 leading neuro-oncology societies, including Koichi Ichimura and Ryo Nishikawa (ASNO), Riccardo Soffietti and Michael Weller (EANO) as well as Susan Chang and Russell Pieper (SNO). The meeting provided an enthusiastic environment for the exchange of ideas among clinical and laboratory scientists involved in the research, diagnosis, care, and treatment of patients with central nervous system tumours.

■ Education Day

The education day on Thursday, November 21, was entitled “From Drug Discovery to Clinic” and reviewed the various aspects of clinical development of agents that comprise preclinical testing, pharmacokinetic and pharmacodynamic considerations, first-in-man studies, and the challenges of phase-0–III clinical trials. Alternate concurrent sessions appraised the specific challenges of clinical design and specific pathways that are being evaluated in the preclinical arena. Also featured was a quality-of-life session with modules focused on palliative care, symptom management, and paediatric quality of life.

■ WFNO Quadrennial and SNO Annual Meeting

The meeting built on the traditional SNO format and presented top-scoring abstracts and meet-the-expert sessions. The scientific meeting began Friday, November 22, with sunrise sessions followed by the start of the first general session. The 4 sunrise sessions were on the following topics: (1) maximal safe resection of glioma – current techniques, (2) paediatric genomics update, (3) innovative QOL programmes in the clinical setting, and (4) metabolic imaging. After the sunrise sessions, the first plenary session started with an official meeting welcome by Dr Mitchel Berger, followed by the Victor Levin Lecture by Monika Hegi and Roger Stupp. Dr Kenneth Aldape then delivered his presidential address followed by the EANO keynote address by Stefan Pfister, MD, PhD, entitled “Translating next-generation diagnostics into next-generation treatment”.

A young investigators luncheon roundtable was held at noon on Friday at which trainees and early-phase independent in-

vestigators participated in informal discussions with senior investigators at roundtables organized into a variety of different areas. Lunch was followed by afternoon concurrent sessions including (1) pathology and genomic and (2) adult clinical sciences. The next set of concurrent sessions included (1) angiogenesis and invasion and (2) paediatric clinical sciences. Friday evening, a special “Townhall Meeting” reviewed the results of the recent international randomised phase-III trials evaluating the use of antiangiogenic strategies for newly diagnosed and recurrent glioblastoma session.

Saturday sunrise sessions featured the following topics: WHO classification of tumours – update (Session 1), overview of GWAS and potential for international collaboration (Session 2), immunological strategies in neuro-oncology (Session 3), asymmetric cell division – avenues for research and clinical applications (Session 4), and joint AAN session: controversies in neuro-oncology (Session 5). After the sessions, a mini-symposium on low-grade gliomas was held and trailed by the ASNO keynote address by Do-Hyun Nam, MD, PhD, entitled “Personalized targeted therapeutics based on the genomic-characterized patient-derived model (Avatar) system”. Up next, award-winning abstracts were presented and they were followed by the SNO keynote address by Frank McCormick, PhD, entitled “New ways of targeting RAS”.

The first-of-its-kind mid-level faculty networking luncheon was held at noon on Saturday with the intent of forming a horizontal mentoring group. After lunch, we witnessed the first set of Saturday afternoon concurrent sessions: (1) cell biology and signalling, (2) molecular epidemiology and biomarkers. The second set of concurrent sessions included (1) paediatric basic and translational research and (2) neurocognitive outcomes and quality of life. A poster session was organised after the oral sessions concluded for the day. That evening, the SNO gala dinner at San Francisco City Hall was the social highlight of the meeting that allowed us to recognise the important service of those who make the meeting possible.

The Sunday, November 24, sunrise sessions included (1) brain metastases, (2) minimising side effects from radiation, (3) tumour-associated epilepsy, and (4) microglia. The sunrise sessions were followed by concurrent meetings on metabolic pathways/stem cells and imaging and RANO update. The last concurrent sessions were (1) preclinical therapeutics and (2) biologic/immunologic therapies followed by the meeting’s adjournment.

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