Decree of the Rector n. 1276 of 04/12/2023

Competition for awarding 1 research grant at the University of Udine

DISCLAIMER:
The official and legally binding call for applications is in Italian only. This document cannot be used for legal purposes and is only meant to provide information in English on the call for applications (Decree of the Rector n. 1276 of 04/12/2023). Please refer to the official call published on: https://www.uniud.it/it/albo-ufficiale

Any change and integration will be made available on the above mentioned web page. Therefore, no personal written communication regarding the examination date and/or competition results shall be provided to applicants.

Annex 1

Competition announcement for the assignment of 1 research grant at the University of Udine, entitled “Novel compounds for glioblastoma therapy: evaluation of the efficacy in in vivo models” SSD: MED/08 (principal investigator, Daniela Cesselli)

Research grant funded by the resources of the project PRIN 2022 - Prot. n. 2022MWE5JY

Art. 1

A selection procedure is hereby launched for the award of 1 research grant at the University of Udine, as identified in Attachment A which constitutes an integral part of the present announcement. The research grant is linked to the research project and is subject and conditioned upon the relative funding. The fellowship may be renewed, in compliance with Art. 22, Law No. 240 of 30 December 2010 (as in the text in force before the implementation of the Conversion Law of the D.L. 36/2022, L. 79/2022), Law No. 11 of 27 February 2015, and the current regulations of the University of Udine for awarding research grants, issued with the Rector’s Decree No. 182 of 31 March 2021. The renewal is subject to the scientific coordinator’s positive assessment of the researcher’s activities, an adequate scientific rationale, and a corresponding financial covering.

The research fellowship does not give rise to any right with regards to accessing University posts.

Any personal communication to candidates related to this selection will be sent exclusively to the email address indicated when registering for the selection, as mentioned in Art. 5.

Art. 2

The research grant described in this competition announcement and the required qualifications to apply for the position are identified in Attachment A. The lack of the admission requirements leads to the automatic exclusion from the competition procedure.

Possession of a PhD or equivalent degree obtained abroad or, only for the interested areas, of a medical specialization accompanied by an adequate scientific production, constitutes a preferential
qualification for awarding the research fellowship of this selection, if it has not been provided as a mandatory requirement.

Candidates in possession of a specialization qualification in the medical area obtained abroad must also attach the recognition decrees issued by the Italian Ministry of Health in order to avail themselves of the qualification of specialist doctor and of the title of surgeon. These recognition decrees are also required if the qualification has already been recognized in another country of the European Union. http://www.salute.gov.it/ProfessioniSanitariePubblico/

For the only purpose of the admission to the competition, the Examining Board (Art. 7) shall assess the equivalence of the qualification obtained abroad, except for the evaluation of the medical specialization qualification to which Article 38 of the Legislative Decree 165/2001 and subsequent modifications and additions, and EU regulations on the matter, shall be applied.

The Examining Board will proceed to the evaluation of the qualification obtained abroad according to the documentation attached to the application form. The Examining Board may exclude the candidate if the submitted documentation does not provide sufficient information for the assessment. Therefore, applicants must enclose all the documentation in their possession relating to their qualification in order to provide the Examining Board with sufficient information for assessment.

Candidates holding a qualification issued by a European Research Area country, if successful, must submit, if not already attached to the application form one of the following options:
- Supplement Diploma in English issued by the competent University.
- CIMEA Certificate of comparability of the foreign qualification, issued by CIMEA (Information Centre on Academic Mobility and Equivalence) via the “diplome” service at https://cimea.diplome.eu/udine/#/auth/login

Candidates holding a qualification issued by a non-European Research Area country, if successful, must submit, if not already attached to the application form one of the following options:
- Declaration of the on-site value of the qualification and the certificate relating to the degree with examinations and grades. A certificate in a language other than Italian or English must be accompanied by an official translation into one of these languages (certified by the competent diplomatic-consular authority or certified by a court in Italy).
- CIMEA Certificate of comparability of the foreign qualification, issued by CIMEA (Information Centre on Academic Mobility and Equivalence) via the “diplome” service at https://cimea.diplome.eu/udine/#/auth/login

If the Supplement Diploma or the statement/attestation of comparability are not available when signing the contract, the applicant must demonstrate that he/she has requested the documentation and submit it as soon as possible.

Any exclusion from the selection procedure due to lack of eligibility requirements, absence of required documents, failure to sign the selection application or submission of the selection application in a manner different from what is provided for in this call for applications will be communicated to applicants exclusively at the email address indicated in the application form.
Art. 3

The research grant referred to in this call for applications cannot be awarded:

a. to employees of Universities and the entities referred to in Article 22, section 1, of Italian Law no. 240 of 30 December 2010 (in the text prior to the reform introduced by Law no. 79 of 29 June 2022);

b. to those who have already been awarded research grants pursuant to Italian Law no. 240 of 30 December 2010 (prior to the reform introduced by Law no. 79 of 29 June 2022) for the maximum period provided by law, even if not continuously, excluding the period in which the grant was used in conjunction with the doctorate, up to the legal term of the relative course;

c. to those who have already benefited from research grants and fixed-term researcher contracts provided for, respectively, in Articles 22 and 24 of Italian Law no. 240 of 30 December 2010 (in the text prior to the reform introduced by Law no. 79 of 29 June 2022), for a total of 12 years, even if not consecutive;

d. to anyone who has a degree of kinship or affinity, up to and including the fourth degree, with:
   - the Rector, the Director General or a member of the Board of Directors of the University of Udine;
   - the scientific supervisor or a professor/researcher belonging to the department or organisation hosting the research grant in question.

The research grant provided for in this call for applications cannot be combined:

a) with scholarships of any kind, except for those granted by Italian or foreign institutions to supplement, by means of stays abroad, the fellow's training or research activities;

b) with other research grants;

c) with an employment relationship, even if part-time, without prejudice to the relevant provisions for employees of public administrations.

The grant awarded under this call for applications is also incompatible with simultaneous attendance at university degree courses, either Bachelor's degree or Master's degree courses, research Doctorates with scholarships and medical specializations, in Italy or abroad.

Art. 4

Applicants must enclose with their application, under penalty of exclusion, the following documents:

a) their professional scientific CV, highlighting the candidate's aptitude for carrying out and implementing the research project (Attachment A);

b) their identity card, their passport or any other identification document1;

c) (for candidates with a foreign qualification only) certification or self-certification of both the academic qualification required for the admission to the selection, and of the exams (with evaluation) took during the period of study abroad, and of any other document that can be useful to the evaluation of the degree by the Examining Board.

Applicants can attach to the application, publications and any other certification considered useful to demonstrate the qualification based on the research program (Attachment A) and to certify any research activity accomplished at public or private institutes (indicating the starting and ending date and the duration).

The documents and qualifications mentioned above must be submitted in Italian or English. Those that are not as requested will not be evaluated. Documents originally written in a language other than

---

1 Please be aware that the residence permit is not an identification document.
Italian or English must come with a translation in Italian or English, that the candidate will do on its own responsibility. The translation can be an abstract concerning the thesis.

Italian and Community candidates wishing to submit qualifications referring to conditions and facts attested by Public Administrations must proceed exclusively with self-certification. Non-EU citizens legally residing in Italy may self-certify only data that can be verified or certified by Italian public bodies. They may also use declarations in lieu when provided for by an international convention between Italy and the declarant's country of origin. Non-EU citizens not residing in Italy cannot self-certify.

Only the qualifications possessed by the candidate on the date the application form is submitted and submitted in accordance with the procedures set out in Article 5 will be assessed.

Failure to submit mandatory documents provided for in this article will constitute grounds for exclusion from the selection.

**Art. 5**

The submission of the applications for the present call starts on January 12, 2024 at 2:00 pm (Italian time) and ends on February 15, 2024 at 2:00 pm (Italian time).

The application to take part in the selection must be completed, under penalty of exclusion, using the appropriate online procedure, available at the link [https://pica.cineca.it](https://pica.cineca.it/). The procedure involves an applicant registration step, for those who do not already have an account, and then an application completion step.

Once completed, the online application must be signed in the manner described in the online procedure (manual signature with attached identity document or digital signature), under penalty of exclusion from selection. The application does not have to be signed if you access the above-mentioned online procedure using your SPID ID.

The qualifications referred to in Article 4 must be attached to the application in .pdf format. Individual .pdf files may not exceed 30MB.

The application for participation in the selection is automatically sent to the University of Udine with the definitive closing of the online procedure.

The University Administration:
- is not responsible if it is impossible to read the submitted documentation in electronic format due to damaged files;
- shall not accept or take into consideration qualifications or documents received in paper form or by any means other than what is specified in this article.

Reference to documents or publications already submitted in connection with other competitions is not allowed.

The Administration is not responsible for any missing document or communication because of inaccurate indication of residence and/or address submitted by the candidate during the application. Also, the Administration is not responsible if the candidate has not communicated changes in this
information, or has communicated them too late. The Administration is also not responsible for any postal or telegraphic problems not attributable to the Administration itself.

Applicants are advised not to wait until the last few days before the closing date to submit their application. The University accepts no responsibility for any malfunctions due to technical problems and/or overloading of the communication line and/or application systems.

**Art. 6**

The selection procedure is held in accordance with the modality indicated in Attachment A.

The test will aim to assess the general preparation, experience and aptitude for research of the candidate. It will consist in the evaluation of the professional scientific curriculum, of the publications and qualifications presented, and of the interview, where foreseen.

**Art. 7**

The Examining board for the competition is identified in Attachment A of the present competition announcement, of which it is an integral part.

At its first meeting, the Examining board shall appoint its President and Secretary, and establish the criteria and methods for evaluating the qualifications and the interview, where foreseen.

The results of the qualifications assessment must be disclosed to applicants during the interview, where foreseen.

The Examining board can award a maximum of 100 points (one hundred out of one hundred) to the selection.

At the end of the evaluation procedure, the Examining board shall formulate the general merit list based on the overall score of each candidate, and draw up the minutes of the whole competition procedure.

Based on the ranking list, the assignment is awarded to candidates who have obtained a minimum overall score of 70/100 (seventy out of one hundred).

The Examining board’s judgement is final.

The ranking list will be made public exclusively through publication on the University's official website.

Applicants will not be notified of the outcome of the evaluation.

Those who do not declare their acceptance of the research grant and do not present themselves at the research centre within the deadline communicated by the latter, even if not formally, shall lose the right to receive it. Exceptions to this term will only be granted in cases of documented force majeure.

The selected candidate will have to undergo any health assessment deemed necessary by the competent doctor and aimed at issuing the assessment of suitability for the specific task according to the protocol of the host structure. The signature of the contract will be possible only after obtaining the judgment of suitability for the specific task by the Azienda Sanitaria Universitaria Friuli Centrale (ASU FC). Before signature of the research grant contract, the candidate awarded of reference research
grant must submit a copy of the vaccination booklet or related certificate, and intradermal reaction – sec. Mantoux (performed in the last 12 months).

Art. 8

The research activity cannot be started before signing the contract defining the terms and conditions of the collaboration.

The activity covered by the research grant must have the following characteristics:

a) it must be carried out as part of the research programme covered by the grant and not be a merely technical support to it;
b) it must have a close connection with the realization of the research program for which the winner of the grant has been awarded the contract;
c) it must be continuous and, in any case, temporally defined, not merely occasional, and in coordination with the overall activity of the University;
d) it must be carried out autonomously, solely within the limits of the programme prepared by the programme supervisor, without predetermined working hours.

The researcher is required to submit a detailed written report on the work carried out and the results achieved, accompanied by the opinion of the scientific supervisor, to the reference organisation at the intervals set out in the contract. The researcher must also submit interim reports and timesheets, if requested by the reference organisation.

Either the fellow or the reference organisation may withdraw from the contract.

The reference organisation may terminate the contract not only in the cases referred to in Article 9, sections 2 and 3, of the "Internal rules for awarding research grants pursuant to law 240 of 30 December 2010" of the University of Udine, but also in the event the research project and therefore the financial coverage on which the research grant is based cease to exist.

With regard to accidental insurance and third-party liability, the provisions of art. 3 c. 5 of the "Internal rules for awarding research grants pursuant to Italian Law no. 240 of 30 December 2010" of the University of Udine, issued by Rector's Decree no. 182 of 31 March 2021, are applied.

The Azienda Sanitaria Universitaria Friuli Centrale (ASU FC), by authorizing with a subsequent deed the access of the selected candidate to its facilities, ensures insurance cover for professional risks and third-party liability in the course of the authorized activity. The insurance policy for accidents and occupational diseases remains at the expense of the selected candidate. In the absence of such policy, the selected candidate will not be allowed to access the facilities of the Azienda Sanitaria Universitaria Friuli Centrale (ASU FC), and it will not be possible to proceed with the signature of the contract.

Art. 9

The following legal dispositions shall apply to the grant referred to in this call for applications:
- for tax matters, the provisions of Article 4 of Italian Law no. 476 of 13 August 1984, as subsequently amended and supplemented;
- for social security matters, the provisions of Article 2(26) et seq. of Italian Law no. 335 of 8 August 1995, as subsequently amended and supplemented;
- for mandatory maternity leave, the provisions of the Italian Ministerial Decree of 12 July 2007;
- with regard to sick leave, the provisions of Article 1(788) of Italian Law no. 296 of 27 December 2006 and subsequent amendments.
During the period of mandatory maternity leave, the allowance paid by INPS according to Art. 5 of the Italian Ministerial Decree of 12 July 2007 is supplemented by the University up to the full amount of the research grant.

The grant will be paid in monthly instalments.

**Art. 10**

The data collected as part of the procedure referred to in Art. 5 are necessary to properly manage the selection procedure, for any subsequent management of the research grant and for purposes related to managing services provided by the University. The University of Udine is the Data Controller. At any time, the data subject may request access, rectification and, depending on the University's institutional purposes, cancellation and restriction of processing or oppose the processing of their data. The data subject can always lodge a complaint with the Italian Data Protection Authority. The complete disclosure is available on the University of Udine website in the "Privacy" section, accessible from the home page [www.uniud.it](http://www.uniud.it) Direct Link: [https://www.uniud.it/it/pagine-speciali/guida/privacy](https://www.uniud.it/it/pagine-speciali/guida/privacy)

**Art. 11**

For all matters not expressly mentioned in this call for applications, refer to the regulations in force on the subject cited in the introduction and to the "Internal rules for awarding research grants pursuant to Italian Law no. 240 of 30 December 2010" of the University of Udine, issued by Rector's Decree no. 182 of 31 March 2021.

**Art. 12**

The procedure supervisor is Dr Sandra Salvador, Head of the Research Services Area of the University of Udine.

The Responsible office at the University of Udine is "Area Servizi per la Ricerca - Ufficio Formazione per la Ricerca", via Mantica n. 31 - 33100 Udine, Italia.

To request information about the call for applications, please complete the following form available on the University of Udine website:
[https://helpdesk.uniud.it/SubmitSR.jsp?type=req&accountID=universityofudine&populateSR_id=42105](https://helpdesk.uniud.it/SubmitSR.jsp?type=req&accountID=universityofudine&populateSR_id=42105)
Novel compounds for glioblastoma therapy: evaluation of the efficacy in in vivo models.

Introduzione
Il glioblastoma multiforme (GBM) rappresenta il tumore primitivo maligno più frequente e aggressivo che colpisce il cervello adulto. Con l’attuale protocollo terapeutico approvato (protocollo Stupp), consistente nella resezione chirurgica seguita da radioterapia e chemioterapia (temozolomide), la prognosi dei pazienti con GBM rimane particolarmente infausta, con una sopravvivenza mediana dei pazienti di circa 14 mesi. Risulta, quindi, di primaria importanza l’identificazione di nuovi bersagli terapeutici, al fine di mettere a punto nuovi protocolli per il trattamento del GBM. Le maggiori difficoltà nel trattamento del GBM sono legate, da una parte, alla capacità dei farmaci di raggiungere il tumore a causa della presenza della barriera ematoencefalica (BBB), dall’altra, alla natura altamente infiltrante del GBM, che rende raramente possibile un intervento chirurgico radicale. Quest’ultima proprietà risiede in una specifica popolazione cellulare, ovvero le cellule staminali tumorali del gliaoma (glioma stem cells, GSCs), responsabili della resistenza alle terapie e causa delle recidive del GBM. Esse, infatti, oltre ad essere resistenti alle radiazioni e ai trattamenti chemioterapici, hanno la capacità di migrare al di fuori del tumore come singole cellule, invadendo il parenchima cerebrale circostante, anche a distanza dalla massa tumorale di origine. Inoltre, crescenti evidenze suggeriscono che la chemioterapia e la radioterapia possano aumentare ulteriormente l’invasione del gliaoma, ostacolando in parte i benefici terapeutici. Infine, è stato dimostrato che il microambiente tumorale, formato da cellule staminali mesenchimali (glioma associated stem cells, GASCs), gioca un ruolo fondamentale nell’insorgenza e nella progressione della massa tumorale. La presenza di un continuo crosstalk cellulare, caratterizzato da una segnalazione pro-sopravvivenza e pro-invasione, suggerisce che la suscettibilità alla chemio e alla radioterapia potrebbe essere aumentata inibendo l’invasione delle cellule di gliaoma. Pertanto,
utilizzare i meccanismi di invasione del glioma come possibile bersaglio terapeutico potrebbe aprire la strada a nuovi trattamenti in grado di invertire la prognosi severa del GBM.

La motilità cellulare si basa sugli stessi meccanismi molecolari sia nelle cellule sane che nelle cellule tumorali del GBM. Infatti, il legame di specifici fattori chemiotattici o pro-migratori ai recettori presenti sulla superficie cellulare promuove l’invasione cellulare mediante l’attivazione di cascate intracellulari a valle, che inducono la riorganizzazione del citoscheletro di actina della cellula bersaglio, con la formazione di filopodi, lamellipodi e podosomi, e la presenza di un coordinamento spazio-temporale della formazione del sito di adesione nella parte anteriore della cellula e rottura nella parte posteriore, per consentire la motilità. Tra le molte vie di segnalazione intracellulari implicate nella migrazione cellulare, un ruolo fondamentale nella regolazione della motilità è svolto dalle Rho GTPasi, piccole proteine presenti all’interno della cellula, che agiscono come “switches molecolari” regolate dal legame con il GTP/GDP e capaci di controllare la rapida e dinamica riorganizzazione del citoscheletro di actina in seguito ad appropriati stimoli. Numerose alterazioni nella cascata di segnalazione delle Rho GTPasi sono state evidenziate in diversi tipi di cancro, compresi i gliomi. Tra i membri maggiormente caratterizzati della famiglia delle Rho GTPasi, associati al rimodellamento del citoscheletro (RhoA, Rac1 e Cdc42), nel presente progetto focalizzeremo la nostra attenzione su Cdc42, responsabile della formazione dei filopodi e della modulazione della polarità cellulare.

Recentemente, Magistrato e collaboratori (CNR-IOM SISSA-Trieste), mediante screening in silico di un’ampia libreria di piccole molecole, hanno selezionato 21 composti come potenziali nuovi farmaci in grado di agire come inibitori reversibili, non covalenti, di Cdc42. Mediante un saggio di migrazione cellulare attraverso una membrana dotata di pori di diametro noto abbiamo potuto selezionare 2 composti, tra i 21 identificati inizialmente, maggiormente efficaci e promettenti (composti #7 e #11 – qui di seguito indicati come C#7 e C#11). Entrambe le molecole hanno infatti mostrato la capacità di inibire di almeno il 40% la migrazione di cellule di glioblastoma (U87 MG) attraverso pori di 3 μm di diametro quando testate mediante saggio Transwell in vitro.

### Obiettivi dello studio


L’obiettivo principale del presente studio, all’interno del quale rientra l’attività dell’assegnista di ricerca, sarà valutare l’efficacia di due nuovi composti (C#7 e C#11) nel ridurre l’invasività delle cellule del glioma in modelli sperimentali in vivo.

A tal proposito, l’assegnista di ricerca sarà coinvolto nella definizione dell’efficacia dei nuovi farmaci identificati per la terapia del glioma attraverso 3 step successivi.

1. **Valutazione della capacità di C#7 e C#11 di attraversare la barriera emato-encefalica (BBB) per raggiungere la sede bersaglio del tumore**

   Nonostante numerose evidenze riportino una parziale disfunzione della BBB nei pazienti affetti da glioma, l’impermeabilità a molte sostanze, e quindi la difficoltà dei farmaci di raggiungere la sede del tumore, rappresenta ancora una delle principali cause del fallimento delle terapie. Pertanto, prima di condurre gli esperimenti in vivo, l’assegnista si occuperà di investigare la capacità di C#7 e C#11 di attraversare la BBB utilizzando un modello in vitro, precedentemente descritto in letteratura, basato sulla co-coltura di tre diversi tipi cellulari (cellule endoteliali, astrocioti e periciti), il quale consente di mimare le caratteristiche della BBB in condizioni fisiologiche. **Risultati attesi:** valutazione della permeabilità della BBB ai composti C#7 e C#11, al fine di stimare la capacità del farmaco di raggiungere il tessuto cerebrale negli studi in vivo.
2. Valutazione della capacità di C#7 e C#11 di inibire la migrazione e l'invasione cellulare in un modello di xenotraapianto in Zebrafish (in collaborazione con il gruppo del Prof. Mantoletti presso l’Università di Trieste)
L'embrione di Zebrafish (Danio rerio) è un modello animale amplamente utilizzato come modello di xenotraapianto nella ricerca sul cancro, e verrà utilizzato nel presente studio come sistema di screening per valutare l'efficacia, in vivo, dei composti C#7 e C#11. In particolare, l'assegnista sarà coinvolto nell'analisi dei potenziali effetti citotossici dei composti e nella valutazione della capacità invasiva di linee cellulari di glioma, trattate o meno con i composti C#7 e C#11. **Risultati attesi:** valutazione della tossicità dei composti C#7 e C#11 e selezione del composto più efficace nell'inibire l'invasione cellulare in un modello in vivo.

3. Valutazione della capacità di C#7 e C#11 di inibire la migrazione e l'invasione cellulare in un modello murino di glioma ortotopico
L'efficacia del composto selezionato nel trattamento del GBM sarà valutata e validata in vivo utilizzando un modello di xenotraapianto ortotopico di GBM nel topo, il quale si basa sull'impianto di cellule tumorali nell'emisfero cerebrale destro (striato) di topi immunocompromessi (SCID-beige/nude), attraverso un apparato stereotassico. Successivamente, gli animali verranno suddivisi in gruppi e sottoposti al trattamento con il composto selezionato (o agli appropriati controlli), nelle dosi e con le modalità determinate mediante analisi della letteratura. Gli animali verranno sacrificati quando saranno evidenti i primi sintomi della malattia (atastia, letargia, perdita di peso) e il cervello murino verrà espiantato per la valutazione istopatologica. Le dimensioni del tumore verranno calcolate dopo l'espianto e le sezioni tissutali verranno analizzate per determinare: a) l'indice proliferativo del tumore; b) la presenza di un fenotipo angiogenico e invasivo; c) la presenza di cellule staminali di glioma umano migrate dal sito di iniezione nel parenchima cerebrale circostante. **Risultati attesi:** valutazione dell'efficacia, in vivo, del composto selezionato nel ridurre la diffusione del tumore.

**Text in English:**

**Introduction**
Glioblastoma multiforme (GBM) represents the most frequent and aggressive primary malignant tumor affecting the adult brain. With the current approved therapeutic protocol (Stupp protocol), consisting of surgical resection followed by radiotherapy and chemotherapy (temozolomide), the prognosis of patients with GBM remains particularly poor, with a median patient survival of approximately 14 months. The identification of new therapeutic targets is therefore of primary importance to develop new protocols for the treatment of GBM. The major difficulties in the treatment of GBM are linked, on the one hand, to the ability of the drugs to reach the tumor, due to the presence of the blood-brain barrier (BBB), and, on the other hand, to the highly infiltrative nature of GBM, which makes radical surgery rarely feasible. The latter feature resides in a specific cell population, namely glioma tumor stem cells (GSCs), responsible for resistance to therapies and GBM relapses. In fact, in addition to being resistant to radiation and chemotherapy treatments, they can migrate outside the tumor as single cells, invading the surrounding brain parenchyma, even at a distance from the tumor mass of origin. Furthermore, growing evidence suggests that chemotherapy and radiotherapy may further increase glioma invasion, partially hindering therapeutic benefits. Finally, it has been demonstrated that the tumor microenvironment, formed by mesenchymal stem cells (glioma associated stem cells, GASCs), plays a fundamental role in the onset and progression of the tumor mass. The presence of continuous cellular crosstalk, characterized by pro-survival and pro-invasion signaling, suggests that susceptibility to chemotherapies and radiotherapy could be increased by inhibiting the invasion of glioma cells. Therefore, using glioma invasion mechanisms as a possible therapeutic target could pave the way for new treatments capable of reversing the severe prognosis of GBM.
Cell motility relies on the same molecular mechanisms in both healthy cells and GBM tumor cells. In fact, the binding of specific chemotactic or pro-migratory factors to the receptors present on the cell surface promotes cell invasion through the activation of downstream intracellular cascades, which induce the reorganization of the actin cytoskeleton of the target cell, with the formation of filopodia, lamellipodia and podosomes, and the presence of a spatio-temporal coordination of the formation of the adhesion site in the anterior part of the cell and rupture in the posterior part, to allow its motility. Among the many intracellular signaling pathways involved in cell migration, a fundamental role in the regulation of motility is played by Rho GTPases, small proteins present inside the cell, which act as "molecular switches" regulated by the binding with GTP/GDP and capable of controlling the rapid and dynamic reorganization of the actin cytoskeleton following appropriate stimuli. Numerous alterations in the Rho GTPase signaling cascade have been highlighted in several cancer types, including gliomas. Among the most characterized members of the Rho GTPase family, associated with cytoskeletal remodeling (RhoA, Rac1 and Cdc42), in the present project we will focus our attention on Cdc42, responsible for the formation of filopodia and the modulation of cell polarity. Recently, Magistrato and collaborators (CNR-IOM SISSA-Trieste), through in silico screening of a large library of small molecules, selected 21 compounds as potential new drugs capable of acting as reversible, non-covalent inhibitors of Cdc42. By means of a cell migration assay through a membrane equipped with pores of known diameter, we were able to select the 2 compounds, among the 21 initially identified, that were most effective and promising (compounds #7 and #11 – hereinafter referred to as C#7 and C #11). Both molecules have in fact shown the ability to inhibit the migration of glioblastoma cells (U87 MG) through pores of 3 μm in diameter by at least 40% when tested using in vitro Transwell assay.

Aims of the study
The objectives and activities of this research grant are part of a broader project, financed under the PRIN project: RESEARCH PROJECTS OF RELEVANT NATIONAL INTEREST - Call 2022. Prot. 2022MWE5JY. "Targeting glioma invasion: further weapons to improve therapeutic efficacy towards glioblastoma (acronym: ARREST)", whose final aim is the development of innovative therapies to block the invasiveness of brain tumors. The main objective of the present study, which includes the research fellow's activity, will be to evaluate the effectiveness of two new compounds (C#7 and C#11) in reducing the invasiveness of glioma cells in vivo experimental models.
In this regard, the research fellow will be involved in defining the effectiveness of the new drugs identified for the therapy of glioma through 3 subsequent steps.

1. Evaluation of the ability of C#7 and C#11 to cross the blood-brain barrier (BBB) to reach the tumor site
Despite numerous evidence reporting a partial dysfunction of the BBB in glioma patients, the impermeability to many substances, and therefore the difficulty of drugs to reach the tumor site, still represents one of the main causes of treatment failure. Therefore, before conducting the in vivo experiments, the fellow will investigate the ability of C#7 and C#11 to cross the BBB using an in vitro model, previously described in the literature, based on the co-culture of three different cell types (endothelial cells, astrocytes and pericytes), which allows mimicking the characteristics of the BBB in physiological conditions. Expected results: evaluation of the permeability of the BBB to compounds C#7 and C#11, to estimate the ability of the drug to reach brain tissue in vivo studies.

2. Evaluation of the ability of C#7 and C#11 to inhibit cell migration and invasion in a Zebrafish xenograft model (in collaboration with Prof. Manfioletti’s group at the University of Trieste)
Zebrafish (Danio rerio) embryo is an animal model widely used as a xenograft model in cancer research, and will be used in the present study as a screening system to evaluate the efficacy, in
vivo, of compounds C#7 and C #11. In particular, the grant holder will be involved in the analysis of the potential cytotoxic effects of the compounds and in the evaluation of the invasive capacity of glioma cell lines, treated or not with compounds C#7 and C#11. Expected results: evaluation of the toxicity of compounds C#7 and C#11 and selection of the most effective compound in inhibiting cell invasion in an in vivo model.

3. Evaluation of the ability of C#7 and C#11 to inhibit cell migration and invasion in a mouse model of orthotopic glioma
   The efficacy of the selected compound in the treatment of GBM will be evaluated and validated in vivo using a mouse orthotopic xenograft model of GBM, which is based on the transplantation of tumor cells in the right cerebral hemisphere (striatum) of immunocompromised mice (SCID - beige/nude), through a stereotaxic apparatus. Subsequently, the animals will be divided into different groups and subjected to treatment with the selected compound (or appropriate controls), in the doses and with the methods determined through literature analysis. The animals will be sacrificed when the first symptoms of the disease are evident (ataxia, lethargy, weight loss) and the murine brain will be explanted for histopathological evaluation. Tumor size will be calculated after explantation and tissue sections will be analyzed to determine: a) the tumor proliferative index; b) the presence of an angiogenic and invasive phenotype; c) the presence of human glioma stem cells that migrated from the injection site into the surrounding brain parenchyma. Expected results: evaluation of the effectiveness, in vivo, of the selected compound in reducing the spread of the tumor.

Struttura dell'Università di Udine presso la quale verrà sviluppata l'attività di ricerca / Department or other structure of the University of Udine where research activities will be carried out:

Dipartimento di Area Medica / Department of Medicine

Tipologia di attività assistenziale prevista in relazione alle esigenze del programma di ricerca / Type of care activity scheduled in relation to the needs of the research program:

- Nessun contatto con pazienti ma solo con dati clinici sensibili. / No contact with patients but only with sensitive clinical data.
- Attività di laboratorio diagnostico senza finalità assistenziale. / Diagnostic laboratory activity without assistance purposes.

Struttura ospedaliera coinvolta / Hospital facility involved:

Azienda Sanitaria Universitaria Friuli Centrale (ASU FC), Presidio Ospedaliero Universitario Santa Maria della Misericordia – Udine, SOC Istituto di Anatomia Patologica.

Importo dell’assegno di ricerca (al lordo oneri carico assegnista) / Total grant gross for the research fellowship:

€ 19.457,28

Durata dell’assegno di ricerca / Duration of the research fellowship “assegno di ricerca”:

12 mesi / months
Finanziamento / Financed by:


Requisiti di ammissione / Minimum qualifications necessary:

- Possesso del titolo di Dottore di ricerca o titolo equivalente conseguito all'estero;
- possesso di un curriculum scientifico professionale idoneo allo svolgimento dell’attività di ricerca contemplata.
- Research doctorate or equivalent qualification obtained abroad;
- professional scientific curriculum suitable for the research activity above mentioned.

Procedura selettiva / Competition procedure:

Valutazione per titoli e colloquio / Evaluation of titles and oral exam.

I risultati della valutazione dei titoli saranno resi noti agli interessati nel corso del colloquio / The evaluation of the qualifications will be disclosed to candidates during the interview.

<table>
<thead>
<tr>
<th>Calendario del colloquio / Calendar of the oral exam</th>
<th>Modalità / Modality</th>
<th>In presenza / On site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data / Date</td>
<td>5 marzo / March 2024</td>
<td></td>
</tr>
<tr>
<td>Ora / Time</td>
<td>10:00 / 10:00 am (Italian time)</td>
<td></td>
</tr>
<tr>
<td>Luogo / Place</td>
<td>Istituto di Anatomia Patologica Azienda Sanitaria Universitaria Friuli Centrale - ASUFC Piazzale S. Maria della Misericordia n. 15, Udine</td>
<td></td>
</tr>
</tbody>
</table>

Per sostenere il colloquio i candidati devono esibire un valido documento di riconoscimento. / Candidates must come to the interview with a valid identity document.

Eventuali variazioni saranno rese note esclusivamente mediante pubblicazione all’albo ufficiale on line dell’Ateneo / Any change will be made public solely through publication on the University web site

[http://web.uniud.it/ateneo/normativa/albo_ufficiale](http://web.uniud.it/ateneo/normativa/albo_ufficiale)
Commissione giudicatrice / Examining Board:

<table>
<thead>
<tr>
<th>Nome e Cognome</th>
<th>Qualifica</th>
<th>SSD</th>
<th>Università</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membri Effettivi / Permanent members</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daniela Cesselli</td>
<td>PA</td>
<td>MED/08</td>
<td>Università degli Studi di Udine</td>
</tr>
<tr>
<td>Antonio Paolo Beltrami</td>
<td>PA</td>
<td>MED/05</td>
<td>Università degli Studi di Udine</td>
</tr>
<tr>
<td>Laura Mariuzzi</td>
<td>PA</td>
<td>MED/08</td>
<td>Università degli Studi di Udine</td>
</tr>
<tr>
<td>Membri Supplenti / Temporary members</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nicoletta Finato</td>
<td>RU</td>
<td>MED/08</td>
<td>Università degli Studi di Udine</td>
</tr>
<tr>
<td>Alessia Cimadamore</td>
<td>RTD</td>
<td>MED/08</td>
<td>Università degli Studi di Udine</td>
</tr>
</tbody>
</table>