Preisverleihung 2025

STIFTUNG
PROFESSOR DR. MAX CLOËTTA

Heft Nr. 53

Dr. Virginie Hamel und Prof. Dr. Paul Guichard
«Advancing medical research through sub-cellular imaging»

Prof. Dr. Nicola Aceto
«Biology and therapeutic targeting of circulating tumor cell clusters»

STIFTUNG
PROFESSOR DR. MAX CLOËTTA

zweiundfünfzigste Preisverleihung

31. Oktober 2025
Zürich

Heft Nr. 53 der Schriftenreihe

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INHALT

VORWORT

Prof. Dr. Fritjof Helmchen
Stiftungsratspräsident

Die Stiftung Professor Dr. Max Cloëtta freut sich, dieses Jahr das wissenschaftliche Wirken von gleich drei Forscherpersönlichkeiten auszuzeichnen. Der Grund dafür ist, dass einer der beiden Cloëtta-Preise geteilt an **Prof. Dr. Paul Guichard** und **Dr. Virginie Hamel** verliehen wird, die an der Universität Genf gemeinsam eine Forschungsgruppe leiten. Mit Hilfe moderner Mikroskopiemethoden untersuchen sie die Struktur von Proteinkomplexen mit zentralen Funktionen innerhalb von Zellen. Dabei verwenden sie neu entwickelte Verfahren der sogenannten Expansionsmikroskopie, bei der man die zu untersuchenden Zellen bzw. ganze Gewebeproben mit Hilfe von Hydropolymeren aufquellen lässt und so das zu untersuchende Objekt um ein Vielfaches physikalisch vergrössert (expandiert). Selbst mit einem einfachen Lichtmikroskop lassen sich an expandierten Proben subzelluläre und molekulare Strukturen mit spektakulärer Auflösung untersuchen. Gestützt auf diese Methode gelang es Prof. Guichard und Dr. Hamel zum Beispiel, die molekulare Zusammensetzung und Architektur von Zentrosomen, die für die Zellteilung entscheidend sind, im Detail zu verstehen. Diese Erkenntnisse sind auch für das Verständnis von Krankheiten wichtig, weil ein gestörter Aufbau von Zentrosomen zu Fehlfunktionen führt, die sich beispielsweise in einer verstärkten Tumorbildung zeigen.

Die Tumorforschung wiederum ist das zentrale Thema von **Prof. Dr. Nicola Aceto,** unserem zweiten diesjährigen Cloëtta-Preisträger. Die Forschung von Prof. Aceto an der ETH Zürich fokussiert sich dabei auf die Prozesse, die an der Ausbreitung von Tumoren im Körper beteiligt sind, das heisst an der Metastasierung. Metastasen entstehen durch die Zirkulation primärer Tumorzellen im Blut. Prof. Aceto konnte zeigen, dass insbesondere die Ausbreitung von verklumpten Zellen (zirkulierenden Tumorzell-Clustern) die Wahrscheinlichkeit der Ablagerung und Metastasierung in sekundären Geweben erhöht. Die Charakterisierung dieser Tumorzell-

Cluster liefert daher wichtige Erkenntnisse über die Prozesse der Metastasenbildung und kann Krebserkennung und Krebsbehandlung in mehrfacher Hinsicht voranbringen. Zum einen können Tumorzell-Cluster als Biomarker dienen, zum Beispiel um das Risiko einer Metastasierung abschätzen zu können. Zum anderen kann der Prozess der Metastasierung durch gezielte Störung oder Auflösung von Tumorzell-Clustern abgeschwächt werden. Erste präklinische und klinische Studien haben hierbei vielversprechende Ergebnisse erzielt.

Mit den Cloëtta-Preisen 2025 werden somit hervorragende Forscherpersönlichkeiten ausgezeichnet, die im Sinne der medizinischen Forschung beispielhaft neue Einsichten in grundlegende molekulare und zelluläre Prozesse in den Zusammenhang mit Krankheitsmustern stellen und dadurch auch neue therapeutische Ansätze eröffnen. Die Cloëtta-Stiftung freut sich, die Preistragenden am 31. Oktober 2025 in besonders würdigem Rahmen feiern zu dürfen. Die Feier findet in der renovierten Semperaula im Hauptgebäude der ETH Zürich statt, einem historisch bedeutsamen Raum, der ein architektonisches Schmuckstück darstellt und nach dreijähriger Restauration in neuem Glanz erstrahlt.

Im Namen des gesamten Stiftungsrates danke ich Stella Vondra, der neuen Geschäftsführerin der Cloëtta-Stiftung für die hervorragende Zusammenarbeit und wünsche Ihnen allen viel Freude bei der Preisverleihung und den Festvorträgen sowie ein wunderbares Raumerlebnis.

Stella Vondra
Geschäftsführerin

Im Jahr 2025 blickt die Stiftung Professor Dr. Max Cloëtta erneut auf ein bewegtes und zugleich erfolgreiches Jahr zurück. Seit ihrer Gründung verfolgt die Stiftung das Ziel, die medizinische Forschung und ihre naturwissenschaftlichen Nachbardisziplinen in der Schweiz und im Ausland nachhaltig zu fördern.

Dies geschieht durch die Auszeichnung exzellenter Forscherinnen und Forscher mit dem Cloëtta-Preis, durch die Förderung junger Talente mittels Forschungsstellen sowie durch das Programm «Klinische Medizin Plus», das gezielt die Verbindung von klinischer Medizin mit den Grundlagenwissenschaften unterstützt. Ergänzend schafft die Stiftung ein Netzwerk, das Wissenschaft, Klinik und Gesellschaft auf nachhaltige Weise verbindet.

Mit der 52. Verleihung des Cloëtta-Preises am 31. Oktober 2025 setzt die Stiftung ihre Mission fort, wissenschaftliche Spitzenleistungen sichtbar zu machen und den Austausch zwischen Grundlagenforschung und klinischer Anwendung zu fördern.

Stiftungsrat

Der Stiftungsrat besteht aus sechs herausragenden Professorinnen und Professoren der Medizin und der Naturwissenschaften von führenden Schweizer Universitäten sowie drei anerkannten Experten aus den Bereichen Finanzen und Recht. Diese breite und interdisziplinäre Expertise gewährleistet die hohe Qualität der Entscheidungsprozesse und die nachhaltige Erfüllung des Stiftungszwecks.

Unser besonderer Dank gilt den Mitgliedern des Stiftungsrates, die ihr Fachwissen und ihre Erfahrung engagiert einbringen, sowie den externen Expertinnen und Experten, deren Gutachten die sorgfältige Auswahl der Preistragenden entscheidend unterstützen.

Cloëtta-Preis

Der Cloëtta-Preis wird 2025 erneut für herausragende wissenschaftliche Leistungen verliehen. Seit seiner ersten Vergabe 1974 zeichnet die Stiftung Professor Dr. Max Cloëtta jährlich exzellente Forscherinnen und Forscher aus, deren Arbeiten die medizinische Forschung entscheidend prägen und erweitern.

Die diesjährigen Preistragenden stehen exemplarisch für die Verbindung von exzellenter Grundlagenforschung mit innovativen Perspektiven für die klinische Anwendung. Mit ihren Arbeiten leisten sie wegweisende Beiträge zur Weiterentwicklung von Medizin und Biowissenschaften und damit zum Fortschritt im Dienste der Gesellschaft.

Unser herzlicher Dank gilt in diesem Jahr den Verantwortlichen der ETH Zürich, die uns in der neu restaurierten Semperaula willkommen heissen. Ebenso danken wir unserer Vertreterin im Stiftungsrat, Prof. Dr. Sabine Werner, für ihre wertvolle Unterstützung bei der Organisation der diesjährigen Preisverleihung.

Forschungsstellen

Seit 1983 vergibt die Stiftung Professor Dr. Max Cloëtta Forschungsstellen an schweizerischen Universitäten, Kliniken und Instituten. Mit diesem Programm wird gezielt der wissenschaftliche Nachwuchs gefördert: Es richtet sich an vielversprechende Talente bis zum Alter von maximal 40 Jahren, die bereits selbständig arbeiten und eine eigene Forschungsrichtung entwickeln. Ziel der Förderung ist es, jungen Forscherinnen und Forschern den entscheidenden Schritt in ihrer akademischen Laufbahn zu ermöglichen und die Phase bis zur Berufung auf eine Professur zu überbrücken.

Die Stipendien werden alle zwei Jahre vergeben und haben sich seit über 41 Jahren als wertvolles Instrument zur Unterstützung des akademischen Mittelbaus in der Schweiz etabliert. Bei der letzten Ausschreibung im Jahr 2024 konnten drei neue Forschungsstellen vergeben werden, die es den ausgewählten Nachwuchswissenschaftlerinnen und -wissenschaftlern

ermöglichen, ihre zukunftsweisenden Projekte in der medizinischen und naturwissenschaftlichen Forschung weiter auszubauen.

2025 förderte die Stiftung Professor Dr. Max Cloëtta die folgenden Forscherinnen und Forscher an Schweizer Universitäten mit Unterstützungsperioden von dreieinhalb bis fünf Jahren:

Dr. Stephan Hirschi, Jg. 1991, Universität Bern, Institut für Biochemie und Molekulare Medizin.
Projekt: «Unravelling the molecular mechanisms of cholesterol and lipid transport in Plasmodium falciparum: Towards discovery of new antimaterials»
Unterstützungsdauer: 1.1.2026–31.12.2030

Dr. Raphael Morscher, Jg. 1987, Universitäts-Kinderspital Zürich, Pädiatrische Onkologie, Hämatologie und Stammzelltransplantation.
Projekt: «Personalized targeting of metabolism in children with cancer (PeTaMeta)»
Unterstützungsdauer: 1.7.2025–30.6.2030

Dr. Mathias Wenes, Jg. 1987, Universität Genf, Medizinische Fakultät
Projekt: «The metabolic-epitranscriptomic link in CD8 T cells»
Unterstützungsdauer: 1.6.2025–31.5.2030

Dr. Lucas Boeck, Jg. 1980, Universitätsspital Basel, Departement für Biomedizin.
Projekt: «Designing sterilising antibiotic treatments through Antimicrobial Single-Cell Testing (ASCT)»
Unterstützungsdauer: 1.10.2023–30.9.2028

Dr. Joel Zindel, Jg. 1986, Universitätsspital Bern, Departement für Viszerale Chirurgie und Medizin.
Projekt: «Mesothelial cell recruitment in injury repair and post-surgical adhesion formation»
Unterstützungsdauer: 1.5.2023–31.7.2025 *(ursprünglich bis 30.4.2028 geplant)*

Dr. András Jakab, Jg. 1985, Universitäts-Kinderspital Zürich, Zentrum für MR-Forschung.
Projekt: «From axons to therapy: Characterizing the connectivity of the human thalamus with 3D multi-scale imaging»
Unterstützungsdauer: 1.10.2020–31.12.2025

Das Beispiel von Dr. Joel Zindel unterstreicht eindrücklich, wie wertvoll das Forschungsstellen-Programm der Cloëtta-Stiftung für den wissenschaftlichen Nachwuchs ist. Mit seiner Berufung auf eine Tenure-Track-Professur am Departement Biologie der ETH Zürich konnte er den entscheidenden Schritt in seiner akademischen Laufbahn vollziehen. Solche Erfolge belegen, dass die Förderung durch die Stiftung nicht nur individuelle Karrieren prägt, sondern auch einen nachhaltigen Beitrag zur Stärkung der biomedizinischen Forschung in der Schweiz leistet.

Klinische Medizin Plus

Seit 2010 vergibt die Stiftung Professor Dr. Max Cloëtta Stipendien «Klinische Medizin Plus». Medizinerinnen und Medizinern werden während oder unmittelbar nach Abschluss ihrer Facharztausbildung Stipendien von drei bis maximal zwölf Monaten für die Absolvierung einer Spezialausbildung an einer renommierten, vornehmlich ausländischen Institution gewährt. 2025 unterstützte die Stiftung Professor Dr. Max Cloëtta folgende Medizinerinnen und Mediziner mit einem Stipendium:

Dr. med. Isabella Bielicki, Jg. 1987, Universitäts-Kinderspital beider Basel (UKBB).
Projekt: «To gain experience in advanced surgical techniques in pediatric colorectal surgery, including minimally invasive approaches, complex reconstructions, and innovative procedures»
Gastinstitution: Children's Hospital Colorado, Aurora, Colorado, USA,
Unterstützungsdauer: 1.8.2025–30.6.2026

Dr. med. Basile Pache, Jg. 1987, Gynäkologische Onkologische Chirurgie und Geburtshilfe, Universitätsspital Lausanne (CHUV).
Projekt: «Fellowship in gynecological oncological surgery»
Gastinstitution: Montréal University Hospital Center (CHUM),

Montréal, Québec, Canada
Unterstützungsdauer: 1.7.2025–30.6.2026

Dr. med. Ioannis Skalidis, Jg. 1992, Kardiologie, Universitätsspital Lausanne (CHUV).
Projekt: «Novel angiography-derived fractional flouresence (FF) techniques for the evaluation of coronary artery physiology at the catheterization laboratory for the treatment decision of angiographically intermediate coronary lesions»
Gastinstitution: Cardiovascular Institute Paris Sud (ICPS), Paris, France
Unterstützungsdauer: 1.12.2024–31.10.2025

Dr. med. Victor Egon Staartjes, Jg. 1997, Neurochirurgie, Universitätsspital Zürich
Projekt: «Clinical fellowship in neurosurgical / minimally invasive spine surgery, including training in advanced intraoperative imaging and navigated surgery, augmented reality-guided surgery; research in artificial intelligence in spinal neurosurgery»
Gastinstitution: Capio Stockholm Spine Center, Löwenströmska Hospital, Stockholm, Sweden
Unterstützungsdauer: 1.7.2025–30.6.2026

Dr. med. Oliver Bichsel, Jg. 1994, Oberarzt i.V., Klinik für Neurochirurgie, Universitätsspital Zürich.
Projekt: «Fellowship in stereotactic and functional neurosurgery»
Gastinstitution: Division of Neurosurgery, University of Toronto, Toronto Western Hospital, Canada
Unterstützungsdauer: 1.7.2024–30.6.2025

Dr. med. dent. Clemens Raabe, Jg. 1990, Dentalchirurg, Faculty Member, Klinik für Dentalchirurgie und Stomatologie, zmk Bern, Universität Bern.
Projekt: «Clinical training in patients with peri-implant inflammatory diseases»
Gastinstitution: Poliklinik für Zahnärztliche Chirurgie & Implantologie, Zentrum für Zahn-, Mund- und Kieferheilkunde, Goethe-Universität,

Frankfurt am Main, Deutschland,
Unterstützungsdauer: 1.4.2024–31.3.2025

Dr. med. Tabea Sutter, Jg. 1991, Resident, Universitätsspital Zürich.
Projekt: «Training in the field of fetomaternal hematology with a specific focus on improvement of peripartal care of patients with hemoglobinopathies»
Gastinstitution: University of Toronto, Princess Margaret Cancer Centre and Mount Sinai Hospital, Toronto, Canada,
Unterstützungsdauer: 1.7.2024–30.6.2025

Dr. med. Manon Vouga, Jg. 1989, Senior Registrar «Cheffe de Clinique», Universitätsspital Lausanne (CHUV).
Projekt: «Foetal therapy and management of Placenta Accreta Spectrum (PAS) disorders»
Gastinstitution: Saint George's University Hospital, NHS Trust, Tooting, London, United Kingdom,
Unterstützungsdauer: 1.5.2024–30.4.2025

Wechsel der Geschäftsstelle

Im Laufe des Jahres 2024 hat unsere Geschäftsstelle den Wechsel zu einem neuen Dienstleister vollzogen und mit Stella Vondra hat die neue Geschäftsführerin ihre Arbeit reibungslos aufgenommen. Im Jahr 2025 lag das Hauptaugenmerk neben der Weiterführung der Geschäftstätigkeit nach dem Wechsel auch auf der Digitalisierung von Kommunikations- und Arbeitsprozessen, um insbesondere die jüngere Generation von Forscherinnen und Forschern noch besser zu erreichen. Neue digitale Werkzeuge, moderne Kommunikationskanäle und ein aktualisierter Webauftritt sollen in Zukunft dazu beitragen, die Sichtbarkeit der Stiftung zu erhöhen und den wissenschaftlichen Austausch zu fördern. Darüber hinaus unterstützt das Team weiterhin engagiert den Stiftungsrat in seinem Bestreben, die Stiftung Professor Dr. Max Cloëtta auch künftig als Brückenbauerin zwischen wissenschaftlicher Exzellenz und medizinischem Fortschritt zu positionieren.

THE CLOËTTA PRIZE 2025
IS AWARDED TO

DOCTOR

VIRGINIE HAMEL

BORN IN 1977 IN FRANCE,

CO-LEADER OF A RESEARCH GROUP IN THE DEPARTMENT OF MOLECULAR AND CELLULAR BIOLOGY AT THE UNIVERSITY OF GENEVA

FOR HER GROUNDBREAKING WORK IN DEVELOPING ULTRASTRUCTURAL EXPANSION MICROSCOPY (U-EXM) AND ADVANCING OUR UNDERSTANDING OF CENTRIOLES AND CILIA, OPENING NEW PERSPECTIVES FOR GENE THERAPIES AND CANCER IMMUNOTHERAPY

ZURICH, 31ST OCTOBER 2025

IN THE NAME OF THE FOUNDATION BOARD:

THE PRESIDENT

THE VICE PRESIDENT

A MEMBER

VIRGINIE HAMEL

CURRICULUM VITAE

Virginie Hamel, PhD

Personal Information

Family status: 3 children, divorced (Hachet)
Nationality: Swiss, French
Address: Mocel, 30 rue quai Ansermet, 1211 Genève, Switzerland
Website: https://mocel.unige.ch/research-groups/guichard-hamel/overview
ORCID ID: http://orcid.org/0000-0001-5092-2343
Google Scholar: https://scholar.google.ch/citations?user=CQMGXiEAAAAJ&hl=en

Career and Education

2021–now	**Lecturer and Group leader** – Molecular and Cellular Biology Department, UNIGE
Since 2020	**Group leader, Scientific collaborator CS2,** Cell Biology Department, UNIGE
2015–2019	**Group leader, Scientific collaborator CS1,** Cell Biology Department, UNIGE
2013–2015	**Research associate,** EPFL/ISREC, Group: P. Gönczy, Lausanne, Switzerland
2005–2012	**Postdoctoral fellow,** EPFL/ISREC, Group: P. Gönczy, Lausanne, Switzerland
2000–2004	PhD thesis, **EMBL** (DE), Group: Ian Mattaj, Heidelberg, Germany
1999–2000	Master Molecular Biology of the Cell – Ecole normale supérieure de Paris **(ENS),** FR
1996–1999	Bachelor Biochemistry – **University Paris VII,** France

Institutional Responsabilities

Since 2025	**Co-founder** of the GenExM service platform, scientific director
Since 2024	**Director** of the Program of continuing education – practicals in labs chemistry and biology
2023–now	**Co-president** of the Equality-Diversity Commission
2023	**Panel member** of professor's nomination committees
2022–now	**Member** of the teaching commission
2021–now	**Panel member** of the DCI Geneva steering committee
2020–now	**Member** of 17 PhD, TAC and Master defenses
2018–now	**Representative** of the biology pole for the Laboratory of Advanced Technologies
2016–2023	**Member** of the Equality-Diversity Commission

Major Grants

2025	**EMBO workshop** on U-ExM
2025	**EMBO conference** on centrosomes
2024–2026	**Foundation Gelbert,** co-recipient (Kostic – Hamel)
2023–2025	**Foundation Pro-Visu,** co-recipient (Kostic – Hamel)
2023	**EMBO workshop** U-ExM course – main applicant
2022–2025	**HUG confirm,** co-recipient (Brochet – Guichard – Hamel)
2022–2025	**Tandem ISREC foundation,** co-recipient (Hamel – Wolf)
2022–2024	**Planetary Biology Project EMBL,** associated team leader
2021–2025	**SNSF project grant** subside, co-recipient (Guichard – Hamel)
2021–2023	**Foundation Pro-Visu,** co-recipient (Guichard – Kostic – Hamel)

Teaching and Supervision of Junior Researchers

Since the inception of my joint laboratory with Paul Guichard, we have supervised 8 master students, 2 bachelor students, 2 technicians, 9 PhD students, 14 post-docs. In addition, my laboratory has trained more than 50 visiting scientists on expansion microscopy techniques.

Since 2024	**Director** of the Program of continuing education – practicals in labs Chemistry and Biology
2024	**Lecturer** of the Histology-pathology for PhD students (8hrs), UNIGE
2022–now	**Lecturer** of the Cytoskeletal course (8hrs) for bachelor and master students
2021–now	**Coordinator and Lecturer** for 1st year bachelor students «Biologie Fondamentale I» BFI (23hrs)
2021–now	**Coordinator and Lecturer** of practical course (Bachelor III) «Expansion Microscopy» (48hrs)
2018–now	**Coordinator** of practical course in laboratories «Biologie Fondamentale I» (20hrs)

Memberships in Panels, Boards

Since 2024	**Review Commons** editorial advisory board
Since 2024	**Mentor** of PhD students in the molecular and cellular department and faculty of medecine
Since 2024	ASCB and LS2 and Biophysical Society BPS member
Since 2024	Member of the UNIGE PhD school Molecular Biosciences
2023	**Co-founder and member** of the association NanoBEAM on advanced imaging

Scientific Reviewing Activities

Grants:	MRC (Medical Research Council, UK), BBSRC (UK), NWO (NL), UK Research and innovation (UKRI, UK, regular reviewer), MRC career development award (UK), Wellcome grant (UK) Remote reviewer for the ERC Starting Grant 2022, ERC Synergy Grant 2023, ERC Advanced Grant 2024
Manuscripts:	*Cell*, *Science*, Science advances, Nature structural molecular biology NSMB, *Nature Methods, FEBS Journal, Review commons, Communications Biology, Current Biology, PLOS genetics, JCS, Nature biomedical engineering, eLife, Life Science Alliance, Dev Cell, JoVe, ACS Nano, Plant Cell, Journal of Cell Biology.*

International Conferences and Seminars (last 4 years)

Invited presentations < 30 at international symposia or institutes

Organization of Conferences and Workshops

2026	**Co-organizer** of the **EMBO centrosomes and centriole** conference, Lausanne, CH
2026	**Co-organizer** of the **EMBO workshop** on expansion microscopy U-ExM, EMBL, Germany
2024	**Main organizer** of the **EMBO workshop** on expansion microscopy U-ExM, EMBL, Germany
2024	**Trainer** for the **EMBO lecture course** «Imaging marine organisms across scales», IT
2024	**Co-organizer** event «Discovering Microscopic Biodiversity in Switzerland», UNIGE
2023	**Co-organizer** of the **EMBL 1st workshop on expansion microscopy,** Heidelberg, Germany
2019–2022	**Co-organizer** of 3 **ExM Workshops,** UNIGE

Public Outreach Activities

2025	**Invited speaker** for the Pint of Science, Geneva, May 2025
2025	Participation in the «Salon de l'innovation», Palexpo, Geneva, April 2025
2025	**Public conference** – Collège de Saussure, Geneva, January 2025
2024	**JCS Scientists to watch** interview series
2024	Interview in the newspaper **«Le monde»** and in **«Nature»** for the expansion microscopy technique
2023	Image contributor for the book «Les cellules, une histoire de vie» de C. Sardet, édition Ulmer
2023	Member of the TREC expedition 1st stop in Roscoff, France
2021	Contributor for art and science exposition **Cell Worlds** https://www.cell-worlds.com/

2022–2025	Participation in the **«Focus career»** event of UNIGE
2024	Participation in the **«Futur en tous genres»** event of UNIGE
Since 2010	Participation in **«La science appelle les jeunes»** – https://sjf.ch/

Publications

As of May 2025 – 64 total publications
Total citations: 3030
H-index: 29 i10-index: 39
https://scholar.google.ch/citations?user=CQMGXiEAAAAJ&hl=en

THE CLOËTTA PRIZE 2025
IS AWARDED TO

PROFESSOR

PAUL GUICHARD

BORN IN 1982 IN FRANCE, CO-LEADER OF A RESEARCH GROUP

IN THE DEPARTMENT OF MOLECULAR AND CELLULAR BIOLOGY AT THE UNIVERSITY OF GENEVA

FOR HIS GROUNDBREAKING WORK IN DEVELOPING ULTRA-STRUCTURAL EXPANSION MICROSCOPY (U-EXM) AND ADVANCING OUR UNDERSTANDING OF CENTRIOLES AND CILIA, OPENING NEW PERSPECTIVES FOR GENE THERAPIES AND CANCER IMMUNOTHERAPY

ZURICH, 31ST OCTOBER 2025

IN THE NAME OF THE FOUNDATION BOARD:

THE PRESIDENT

THE VICE PRESIDENT

A MEMBER

PAUL GUICHARD

CURRICULUM VITAE

Paul Guichard, PhD

Born in Angers, France, on July 23th, 1982 (42 yo)
Engaged, 3 children (7 yo – 10 yo – 21 yo).
Nationality: French
Phone: 076 296 92 52
Address: Rue de Carouge 76, 1205 Geneva
Website: https://mocel.unige.ch/research-groups/guichard-hamel/overview
ORCID ID: orcid.org/0000-0002-0363-1049
Web of Science ResearcherID: AAL-8603-2020
Google Scholar: https://scholar.google.com/citations?user=fgfaEJIAAAAJ&hl=fr
Bluesky: https://bsky.app/profile/centriolelab.bsky.social

Career and Education

Oct 2021–	**Group leader and Associate Professor,** Cell Biology Dept, UNIGE, Switzerland
2019–2021	**Group leader and Assistant Professor tenure track,** Cell Biology Dept, UNIGE, Switzerland
2015–2021	**Group leader and Assistant Professor,** SNSF Prof. – Cell Biology Dept, UNIGE, Switzerland
2011–2015	Post-Doctoral Fellow – EPFL, Switzerland – Group: P. Gönczy
2008–2012	**Consultant** – Sanofi-Pasteur, Lyon, France
2010–2011	**Post-Doctoral Fellow,** Institut Curie, Paris, France – Group: S. Marco
2007–2010	**PhD thesis** – Sorbonne University and Institut Curie, Paris, France – Group: S. Marco & A. M. Tassin
2006–2007	**Pasteur Course** in biochemistry – Institut Pasteur, France

Major Grants and Awards

2022	CONFIRM – grant Fondation privée des HUG (Brochet – Hamel – Guichard)
2022	**ERC Consolidator Grant**
2022	**Friedrich Miescher Award 2022**
2021–2025	SNSF project grant (co-lead with Dr. Virginie Hamel)
2020–2024	**EMBO Young Investigator (YIP)**
2017–2022	**ERC Starting Grant**
2016	SNSF R'equip 3D cryoEM (co-applicant)
2015–2021	**SNSF Professorship**

Scientific and Administrative Activities

Current activity

Since 2024	**Co-director** of the Molecular and Cellular Biology Department
Since 2024	Chair of the Scientific Steering Board of the Dubochet Center for Imaging
Since 2023	Co-founder and member of the association NanoBEAM for advanced imaging EMBL-TREC
Since 2022	Member of evaluation committee – SNSF Advanced Postdoc.Mobility
Since 2022	Editorial Board member of Journal of Structural Biology (JSB and JSBx)
Since 2022	Member of the Scientific Steering Board of the Dubochet Center for Imaging (DCI Lausanne)
Since 2021	President of the Scientific Steering Board – Geneva cryo-microscopy center (DCI Geneva)
Since 2016	**Co-founder and co-director of the Cryo-EM facility DCI Geneva**
Since 2016	Member of the Scientific Advisory Board – Electron Microscopy Facility, University of Lausanne
Since 2016	Member of the Steering Committee of the Bioimaging facility – University of Geneva

Since 2016 Member of 28 Thesis Advisory Committee
Since 2016 Jury member of 14 PhD thesis exams
Since 2019 Jury member of 2 HDR

Current Teaching Activities (last 5 years)

2021–now Biologie fondamentale (BF1) – **22 h**
Origins of life, energy metabolism, DNA, replication, transcription, translation, cell compartments, intracellular transport and protein secretion, protein structure
2020–now **Co-coordinator** and **Instructor** of practical course (Bachelor III) on photonic microscopy and expansion microscopy – **48 h**
2018–now Master course «Shaping the cell», University of Lyon, France – **2 h**
2017–now Master: Principles of Cellular and Molecular Biology – **6 h**
2016–now International PhD program in Life Sciences (Geneva) – **4 h**

Supervision of Junior Researchers

9 former post-doctoral fellows and 5 current
5 former PhD students and currently 3 PhD students, 7 Master students

International Conferences – Workshops – Seminars

Invited presentations > 60 at international symposia or institutes,

Organisation of Workshops in Expansion Microscopy

Training in Expansion Microscopy

April 2026 EMBO Workshop – Ultrastructure Expansion Microscopy: From Cells to Tissue, EMBL, Heidelberg
April 2024 EMBO Workshop – Ultrastructure Expansion Microscopy, EMBL, Heidelberg

April 2023	EMBL Expansion Microscopy Workshop, EMBL, Heidelberg
Fall 2021	SNSF Scientific exchange – sabbatical of Prof. C. Morrison in my lab to implement U-ExM on DT40
Sept 2019	ExM workshop organization – University of Geneva, Sept 2019 10 participants from institutes all over Europe such as the CRG Barcelona, Gulbenkian Institute or Oxford University
Jan 2019	ExM workshop organization – University of Geneva, Jan 2019 10 participants – UNIGE (CMU and Faculty of Science)
Since 2019	In addition to the ExM workshops we are organizing, we are helping or training several groups to implement U-ExM in their laboratory: Pertz lab (UniBe), Ochsenreiter (UniBe), Azimzadeh lab (Monod Insitute, Paris), Tardieux and Arnoult Labs (IAB, Grenoble), Chretien Lab (IGDR, Rennes), Absalon Lab (Indiana University, USA), Dvorin Lab (Harvard Medical School, USA), Lamy Lab (Campus Biotech, UNIGE), Gonczy Lab (EPFL), Tassin Lab (I2BC, Gif-sur-Yvette), Loewith Lab (UNIGE), Vaughan Lab (Oxford Brookes Univ.), Decelle lab (CNRS, Grenoble), Ulm Lab (UNIGE), Dudin Lab (EPFL), Huber Lab (UNIGE), Castets Lab (UNIGE), Dey Lab (EMBL), Banterle Lab (EMBL), Schwab Lab (EMBL), Delous Lab (UNIGE), Musacchio Lab (MPI, Germany), Pilhofer Lab (ETH, Switzerland), Balestra Lab (Cabimer, Sevilla). Kitagawa Lab (Uni. Tokyo, Japan), Institut Imagine
2020–2021	Serial Volume Editor with Dr. Hamel. Method in cell biology: **Expansion Microscopy for Cell Biology** https://www.elsevier.com/books/expansion-microscopy-for-cell-biology/guichard/978-0-12-820807-6

Outreach

2025	Participation in the «Salon de l'innovation», Palexpo, Geneva, April 2025
2025	**Public conference** – Collège de Saussure, Geneva, January 2025
2024	Interview in the newspaper **«Le monde»** and in **«Nature»** for the expansion microscopy technique
2023	Image contributor for the book «Les Cellules – Une histoire de la vie», Editions Ulmer
2022	Contributor for art and science exposition Cell Worlds https://www.cell-worlds.com/
2016–now	«La science appelle les jeunes» – Swiss Youth in Science https://sjf.ch/

Miscellaneous

Since 2020	Member of LS2 (Life Science Switzerland)
2020	Research Integrity Certificate, Biomedical Sciences – Epigeum Online Course System

Publications

As of May 2025 – 64 total publications
Total citations: 3548
H-index: 34
i10-index: 49
https://scholar.google.fr/citations?user=fgfaEJIAAAAJ&hl=en

RESEARCH OUTPUT LIST

Publications in international peer-reviewed scientific journals (selection of the 10 most relevant publications)

Please see the full list of publications here:

Virginie Hamel: https://scholar.google.com/citations?user=CQMGXiEAAAAJ&hl=en
Paul Guichard: https://scholar.google.com/citations?user=fgfaEJIAAAAJ&hl=en

1. Time-series reconstruction of the molecular architecture of human centriole assembly

   Laporte M.H., Gambarotto D., Bertiaux E., Bournonville L., Louvel V., Nunes J.M., Borgers S., Hamel V.* and Guichard P.*
   Cell – April 2024
   - https://www.unige.ch/medias/en/2024/la-genese-de-notre-squelette-cellulaire-image-par-image
   - https://www.myscience.ch/en/news/2024/the_genesis_of_our_cellular_skeleton_image_by_image-2024-unige
   - https://www.eurekalert.org/news-releases/1040670?language=english

2. Fine-tuning FAM161A gene augmentation therapy to restore retinal function.
   Arsenijevic Y., Chang N., Mercey O., El Fersioui Y., Koskiniemi-Kuendig H., Joubert C., Rivolta C., Sharon D., Guichard P., Hamel V., Kostic C.
   EMBO Molecular Medicine – March 2024
   - https://blog.ophtalmique.ch/2024/03/20/espoir-retinite-pigmentaire/

3. Gene augmentation of LCA5-associated Leber congenital amaurosis ameliorates bulge region defects of the photoreceptor ciliary axoneme
   Faber S., Mercey O., Junger K., Garanto A., Ueffing M., Collin R.W.J., Boldt K., Guichard P.*, Hamel V.*, Roepman R.*
   JCI Insight. April 2023. https://doi.org/10.1172/jci.insight.169162.

4. In situ architecture of the ciliary base reveals the hierarchical assembly of IFT trains
   Van den Hoek H., Klena N., Alvarez G., Jordan MA, Schaffer M., Erdmann PS. Wan WN, Plitzko JN, Baumeister W., Pigino G.*, Hamel V.*, Guichard P.*, Engel BD.*
   Science – July 2022. https://doi.org/10.1126/science.abm6704

5. The connecting cilium inner scaffold provides a structural foundation to maintain photoreceptor integrity
   Mercey O., Kostic C, Bertiaux E., Giroud A., Sadian Y., Chang N., Arsenijevic Y., Guichard P.*, Hamel V.*
   PLOS Biology – June 2022. https://doi.org/10.1371/journal.pbio.3001649
   - https://www.lematin.ch/story/decouverte-romande-pour-esperer-soigner-une-grave-maladie-de-lil-225149132379
   - https://www.news-medical.net/news/20220617/New-discovery-could-lead-to-the-development-of-therapeutic-approaches-for-retinitis-pigmentosa.aspx?alm_mvr=0

6. Visualizing the native cellular organization by coupling cryo-fixation with expansion microscopy (Cryo-ExM)
   Laporte M.H., Klena N., Hamel V* and Guichard P*.
   Nature Methods. Jan 2022. https://doi.org/10.1038/s41592-021-01356-4

7. Expansion Microscopy provides new insights into the cytoskeleton of malaria parasites including the conservation of a conoid structure.
   Bertiaux-Lequoy E., Balestra A., Bournonville L., Brochet M. *, Guichard P.*, Hamel V.*
   PLOS Biology. March 2011. https://doi.org/10.1371/journal.pbio.3001020
   - Commentary: «A conoid ring unites them all»

8. A helical inner scaffold provides a structural basis for centriole integrity.
   Le Guennec M, Klena N, Gambarotto D, Laporte M, Tassin A-M, Van den Hoek H, Erdmann P.S, Schaffer M, Kovacik L, Borgers S, Goldie K.N, Stahlberg H, Bornens M, Azimzadeh J, Engel B.D *, Hamel V* and Guichard P*.
   Science Advances. Feb 2020. https://doi.org/10.1126/sciadv.aaz4137
   - Quarterly picks from AAAS Science Translational Medicine

9. Flagellar microtubule doublet assembly in vitro reveals a regulatory role of tubulin C-ter tails.
   Schmidt-Cernohorska M., Zernov I., Steib E., Le Guennec M., Achek R., Demurtas D., Mouawad L., Lansky Z., Hamel V* and Guichard P.*
   Science. 2019 Jan 18. https://doi.org/10.1126/science.aav2567

10. Imaging cellular ultrastructures using expansion microscopy (U-ExM).
    Gambarotto D., Zwettler F. U. , Cernohorska M., Fortun D., Borgers S., Heine J., Schloetel J. G., Reuss M., Unser M., Boyden E.S., Sauer M.*, Hamel V.* and P. Guichard*
    Nature methods. 2019 Jan 16. https://doi.org/10.1038/s41592-018-0238-1

ADVANCING MEDICAL RESEARCH THROUGH SUB-CELLULAR IMAGING

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1. Introduction

Seeing is believing. This adage has long guided the pursuit of knowledge in biology, where progress has depended as much on the development of new ways of seeing as on the formulation of new ideas. From van Leeuwenhoek's early lenses revealing the existence of cells and microbes, to the electron microscopes of the mid-20th century, which unveiled the hidden intricacy of organelles and proteins, each advance in imaging has opened new frontiers. In cell biology especially, visualization has not been merely confirmatory; it has been transformative, enabling the direct observation of structures and processes that had only been hypothesized. Today, the cell is not just a conceptual unit of life, but a richly imaged landscape whose features continue to sharpen as resolution improves.

Among the discoveries illuminated by imaging, the centriole stands as a landmark example. First identified by Theodor Boveri in the late 19th century, centrioles appeared under the microscope as paired cylindrical structures within the centrosome. Through imaging work, Theodor and Marcella Boveri inferred their role in orchestrating cell division, laying the groundwork for a century of exploration into centrosomes, spindle formation, and cell polarity ***(Figure 1)***[1, 2]. Importantly, they were among the first to propose a link between abnormal centrosome activity and cancer development. They observed that cells with multiple centrosomes frequently underwent aberrant mitosis, resulting in unequal chromosome segregation and aneuploidy ***(Figure 1B–C)***[3–5]. Though initially met with skepticism, Boveri's hypothesis of centrosome-driven tumorigenesis has since been validated. Modern studies confirm that centrosome amplification is a common feature in diverse cancers, driving chromosomal instability, structural abnormalities, and tumor evolution[6].

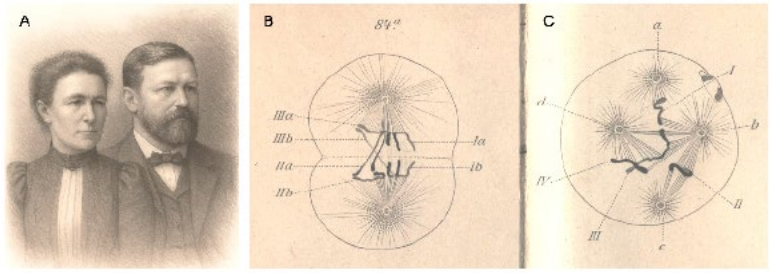

Figure 1. Theodor and Marcella Boveri and their pioneering work on centrosomes, cell division, and cancer theory. *(A) Portrait of Theodor Boveri (1862–1915) and Marcella O'Grady Boveri (1863–1950), both influential biologists. Marcella was one of the first American women to study biology in Europe and collaborated closely with Theodor. (B–C) Original drawings from Theodor Boveri illustrating centrosomes and spindle apparatus organization during cell division. His meticulous observations of abnormal centrosome numbers and chromosomal missegregation in early embryonic development led him to propose that chromosomal imbalance and centrosomal dysfunction could drive uncontrolled cell proliferation. This formed the basis of his influential theory linking chromosomal abnormalities to cancer, which remains foundational in cancer biology today. Pictures B and C are from the «Würzburg Virtual Boveri Library».*

Nowadays, in biomedical research, the disruption of centrosome function has now emerged as a hallmark of cancer biology[7]. Centrosome amplification induces multipolar mitoses and aneuploidy, fueling malignant progression. Advances in high-resolution imaging have further revealed that at the heart of the centrosome lie centrioles, highly ordered cylindrical structures composed of nine triplet microtubules ***(Figure 2A, B)***. These centrioles serve as templates for another crucial organelle: the cilium, hair-like projections that extend from the surface of many eukaryotic cells, broadly classified into motile and non-motile (primary) types ***(Figure 2C)***. Motile cilia are involved in fluid movement across epithelial surfaces, such as in the respiratory tract, while primary cilia serve as sensory organelles that regulate key signaling pathways including Hedgehog, Wnt, and PDGF[8, 9]. Like the centrosome, the cilium plays a vital role in maintaining cellular homeostasis, and its dysfunction contributes to disease. Disruption in ciliary structure or function has been implicated in a range of disorders, often referred to as ciliopathies: from polycystic kid-

ney disease to retinal degeneration, and is increasingly recognized in cancer, where loss or alteration of cilia can dysregulate growth signals and promote malignancy[10, 11]. Moreover, recent discoveries on the centrosome–cilia-cell cycle axis highlight its pivotal role in tumor biology and its potential as a therapeutic target[12, 13]. Together, these insights underscore how imaging-driven discoveries of cellular organelles continue to reshape our understanding of cancer development and progression.

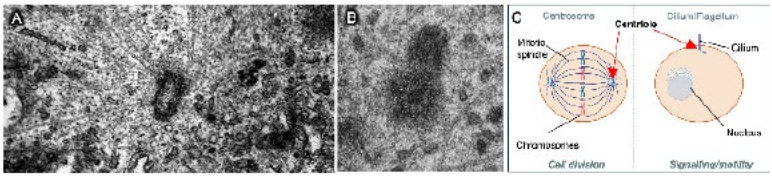

Figure 2. Ultrastructure and functions of the centriole. *(A, B) Transmission electron microscopy (TEM) images showing centrioles in mammalian cells. (A) Centriole within the centrosome, nucleating cytoplasmic microtubules that radiate outward. (B) The cylindrical arrangement of microtubules triplets and their nine-fold symmetry organization can be observed. (C) Schematic representation of centriole functions. Left: centrioles organize the centrosome, which nucleates the mitotic spindle to ensure proper chromosome segregation during cell division. Right: centrioles act as basal bodies to template cilia or flagella, essential for cell signaling and motility.*

Centrioles therefore lie at the heart of two fundamental domains of cell biology, centrosome and cilia biology. These highly conserved organelles are cylindrical, microtubule-based structures measuring approximately 250 nm in diameter and 450 nm in length[14]. Owing to their sub-micrometric dimensions, centrioles have long posed a challenge for detailed structural and functional studies. For many years, limitations in imaging techniques restricted our ability to dissect their molecular composition and to fully appreciate their biological roles. Recent breakthroughs in advanced microscopy, however, have radically transformed our understanding of centrioles and cilia. In particular, the development of electron cryo-microscopy and super-resolution imaging methods and, more recently, expansion microscopy, has provided unprecedented insights into their nanoscale architecture and dynamic functions. These approaches

have uncovered previously hidden structural details, revealed unexpected molecular interactions, and highlighted new, crucial roles for centrioles in both cellular organization and disease.

Here, we will focus on the research we have conducted over the past decade to deepen our understanding of centriolar biology. We will describe how this work has led us to optimize expansion microscopy techniques, enabling us to visualize the molecular architecture of centrioles and cilia with exceptional clarity. Furthermore, we will highlight how these advances are being translated into biomedical research applications, ranging from retinal disease to cancer, highlighting the translational potential of this emerging imaging technology.

2. Centrioles: Decoding a structural mystery

Centrioles are evolutionarily conserved organelles characterized by a distinct ultrastructure composed entirely of protein assemblies and devoid of membranes. The complex architecture of this macromolecular assembly was first revealed in the 1960s using transmission electron microscopy (TEM), which identified their hallmark nine-fold radial symmetry and microtubule triplet arrangement[15, 16]. Over the following decades, advances in conventional electron microscopy and serial-sectioning provided progressively higher-resolution views and their relationship to the pericentriolar material (PCM)[17–19]. From the 90s onwards, biochemical, genetic, and molecular studies further complemented these structural observations by identifying key centriolar components and their role in templating centriole assembly, duplication, and ciliogenesis[20–27]. More recently, cryo-electron microscopy approaches, including cryo-tomography of isolated centrosomes and *in situ* cryo-tomography, have enabled a better understanding of centrioles architecture ***(Figure 3)***[28–35].

Centrioles are barrel-like structures, composed of microtubule triplets arranged in a characteristic 9-fold radial symmetry ***(Figure 3B)***[36]. The microtubule blades consist of one complete A-microtubule and two incomplete B- and C-microtubules ***(Figure 3C)***. Along the centriole's longitudinal axis, microtubule triplets are found in the proximal and central regions, tapering into microtubule doublets toward the distal end.

Moreover, centrioles are structurally polarized along their long axis and can be divided into three distinct regions: 1/a 150 nm proximal region, 2/a 250–300 nm central core region, and finally 3/a 50 nm distal region. Each segment displays unique structural features and contributes to the overall polarity along the proximal-to-distal axis of the centriole ***(Figure 3)***.

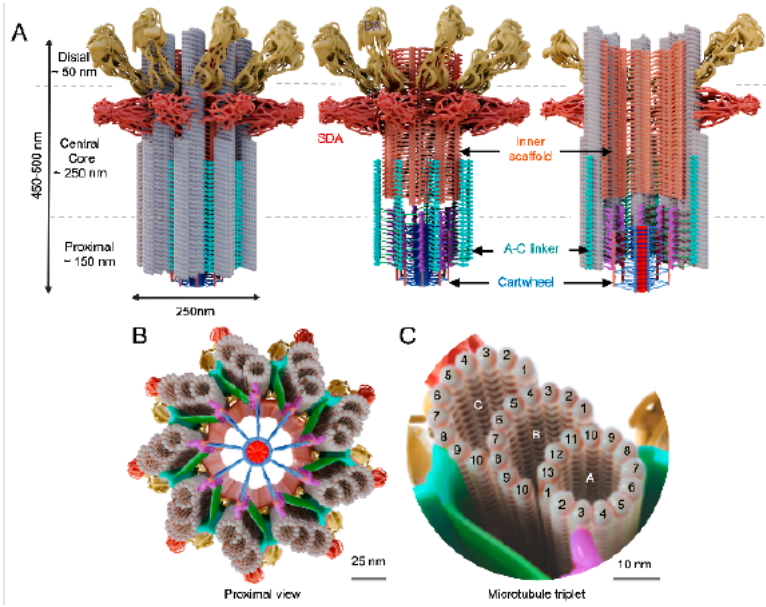

Figure 3: Overview of the centriole's architecture. *(A) Model of a centriole displaying its major structural sub-elements, as a full view with microtubules (left), only the non-microtubule structures (middle) and its longitudinal section (right). Cartwheel, blue; CID, red; D2 density, orange; pinhead, magenta; triplet base, green; A-C linker, cyan; inner scaffold, orange; subdistal appendage, dark red; distal appendages; yellow; tubulin, gray. (B) View of a centriole from the proximal side, revealing the nine-fold symmetry. (C) Close-up of a microtubule triplet. From Le Guennec et al, Current Opinion in Structural Biology 2021*[36].

In the proximal region resides the cartwheel structure, which is essential for centriole duplication and responsible for establishing the organelle's characteristic nine-fold symmetry. This cartwheel is connected to the microtubule triplets via the pinhead structure. Adjacent microtubule triplets are themselves linked by the A-C linker, a structure which also connects to the cartwheel through the triplet base element, making a coherent ensemble in the proximal end. Using cryo-electron tomography (cryo-ET), our laboratory has examined centrioles from four evolutionarily diverse species: isolated centrioles from *Paramecium tetraurelia, Naegleria gruberi,* and human cultured cells, as well as *in situ* centrioles from *Chlamydomonas reinhardtii* cells prepared by cryo-FIB milling. We notably identified the cartwheel's structural unit, composed of three pairs of rings from which spokes extend toward the pinhead structure. We also mapped the boundaries of key sub-elements in this proximal area, particularly the triplet base, which connects the cartwheel to the A-C linker. Moreover, our team and others have characterized the A-C linker architecture across multiple species using cryo-ET. This work showed that the A-linker interacts with the A-microtubule via a density known as the trunk, while the C-linker, comprising two arms (A and B), contacts the C-microtubule, linking adjacent microtubule blades[34]. Interestingly, although the A-C linker structures are largely conserved across the studied species, we observed structural differences between them.

In the central core region, where a Y-shaped linker density connecting the A- and B-microtubules at the so-called inner junction was initially described in *Chlamydomonas*[29], our laboratory discovered a structure that we named «inner scaffold». This scaffold, formed by connected Y-shaped linkers is lining the inner side of the microtubule triplets and doublets, and spans about 70% of the centriole's length, approximately 300 nm long and 150 nm in diameter in humans ***(Figure 3)***[37]. We also showed that the centriole inner scaffold adapts to mechanical stress, maintaining microtubule triplet (MTT) cohesion even under compression. In *P. tetraurelia*, the scaffold withstood deformation better than the proximal A-C linker, preserving structural integrity despite substantial MTT damage proximally. Moreover, comparative analyses across species revealed that the inner scaffold realigns MTT geometry in the central/distal regions, where the MTT twist angle stabilizes, suggesting a conserved role in pre-

paring centrioles for cilia extension. From these analyses, we propose that the inner scaffold plays a crucial role in maintaining centriole stability by holding the microtubule triplets together across the central region[37].

Altogether, using cryo-electron microscopy, the overall architecture of centrioles and their sub-elements has been identified ***(Figure 3)***[36]. However, this technique does not currently allow for the precise assignment of proteins within the observed densities[38]. Unlike in cilia, where single-particle analysis (SPA) has been successfully employed to resolve the composition of macromolecular complexes[39], this approach remains largely unfeasible for centrioles. Its application to centriolar structures is only beginning to emerge. Gaining a molecular-level understanding of the composition of these structural elements is essential if we are to clarify their functional roles and uncover how their disruption contributes to disease conditions.

3. Nanoscale protein mapping and beyond: Expansion microscopy to the rescue

Although recent advances in cryo-electron tomography (cryo-ET) and image processing have greatly improved our understanding of centriolar architecture, their precise molecular composition remains largely unresolved. This limitation arises from intrinsic constraints of cryo-tomography. While modern cryo-EM methods can achieve sub-nanometer resolution, even resolving individual atoms and enabling *de novo* identification of proteins within molecular complexes, this level of detail is only attainable for assemblies of a few hundred kilodaltons[38]. In contrast, the centriole is a macromolecular complex of several giga Daltons[40]. Consequently, at the scale of centrioles, cryo-microscopy cannot reach atomic resolution, leaving the molecular identity of structural sub-assemblies unknown.

An alternative strategy, employed for decades in cell biology, is to localize proteins of interest either by generating specific antibodies or by tagging them with fluorescent reporters such as the Green Fluorescent Protein (GFP). The distribution of these fluorescent proteins, or of antibody-fluorophore conjugates, can then be visualized using fluores-

cence microscopy. This approach has long enabled both visualization of the cell interior and tracking of protein localization. However, fluorescence microscopy is fundamentally limited by a maximum resolution of ~200 nm, a physical constraint defined by Ernst Abbe in the 19th century and known as the diffraction limit[41]. Since centrioles measure approximately 250 nm in diameter and 450 nm in length, their dimensions fall at the boundary of this limit. To localize proteins at the nanometer scale and correlate cryo-tomographic structures with molecular composition, methods that surpass the diffraction barrier are required. This need is met by the development of super-resolution (SR) microscopy, a new class of techniques that overcome the diffraction limit[42]. Over the past decades, SR microscopy has provided powerful tools for visualizing subcellular structures and protein distributions at unprecedented resolutions. These techniques include methods such as Structured Illumination Microscopy (SIM, ~120 nm)[43], Stimulated Emission Depletion (STED, ~40 nm)[44], and Single-Molecule Localization Microscopy (SMLM, ~20 nm)[45]. In the centrosome and cilia fields, these methods have enabled accurate localization of proteins within these organelles, revealing, for instance, the molecular architecture and distribution of proteins localizing outside the centriole barrel, at the level of pericentriolar matrix or the appendages[46–51]. Notably, an SMLM study of 16 centriole distal-end proteins uncovered distinct blade and matrix functional components, providing unprecedented molecular insights into distal-end organization[52–54].

However, despite these important advancements, SR techniques still fall short of providing the ultrastructural resolution of electron microscopy when imaging centrioles. Several factors contribute to this limitation. One key challenge is the high molecular density of the centriole, like other organelles, which restricts antibody accessibility to many epitopes. In addition, antibodies used for immunolabeling are relatively large, typically 10–15 nm in length, which introduces a spatial offset between the target protein and the attached fluorophore, a phenomenon known as linkage error[55]. This displacement imposes a fundamental limit on the achievable resolution of SR approaches. Furthermore, the implementation of SR microscopy often demands highly specialized instrumentation, optimized sample preparation, and advanced expertise in data acquisition and computational analysis, factors that restrict its accessibility and wide-

spread application. Therefore, the development of alternative SR strategies will be essential to probe molecular architectures at the nanoscale and to fully unravel the organization of complex cellular organelles such as centrioles.

In 2015, the group of Edward Boyden (MIT, USA) developed an innovative SR method named expansion microscopy (ExM)[56]. Its underlying principle, in opposition to the rest of SR methods that increase the resolution of the microscope, relies on the physical expansion of the biological specimen embedded in a swellable hydrogel containing the super-absorbent sodium acrylate ***(Figure 4A–C).*** This revolutionized SR imaging making it accessible for everyone using conventional microscopes that are readily accessible by scientists. The original ExM protocol involves labeling the sample with modified fluorescent DNA probes that are attached to a polymer[56]. This is followed by a strong proteinase treatment to degrade proteins, enabling the embedded sample to expand freely in three dimensions. As a result, only the fluorescent moieties remain covalently bound to the hydrogel and are physically separated during polymer expansion, leading to an approximately 4.5-fold isotropic enlargement of the fluorescent pattern, allowing to reach a resolution of 60 nm with a regular fluorescent microscope. Quickly thereafter the method, still based on pre-expansion labeling, was modified allowing the anchoring of proteins directly to the gel and the use of conventional reagents such as primary and secondary antibodies instead of these custom DNA probes[57]. Another ExM-based method utilizing post-expansion labeling was developed and named MAP (Magnified Analysis of the Proteome) ***(Figure 4C)***[58]. In contrast to the original ExM protocol, which uses proteinase digestion, MAP relies on the denaturation of cross-linked proteins embedded within the swellable hydrogel. This enables the direct expansion of the biological specimen while preserving protein content. Immunofluorescence labeling is then performed after expansion. Notably, this approach induces a decrowding effect, improving epitope accessibility, enhancing antibody binding, and reduce the linkage error proportionally to the expansion factor[55].

To investigate the structural subcomponents of the centriole, our laboratory developed and optimized a protocol compatible with super-resolution imaging, which we termed U-ExM (Ultrastructure Expansion Microscopy). This method builds on the principles of the MAP protocol[59]. In brief, proteins within a biological sample are anchored in a polyacrylamide/sodium acrylate hydrogel, denatured, and subsequently expanded by immersion in pure water ***(Figure 4C).*** Immunofluorescence labeling is then performed post-expansion, enabling super-resolution imaging of proteins and organelles such as centrioles using standard optical microscopes ***(Figure 4D).*** To assess the isotropy of expansion achieved with U-ExM, we used isolated *Chlamydomonas* centrioles, whose dimensions are well characterized, as molecular rulers[59,60]. Crucially, we demonstrated that U-ExM preserves centriole ultrastructure, clearly revealing their characteristic nine-fold symmetry. When combined with STED microscopy, U-ExM uncovered features such as centriole chirality and achieved resolutions comparable to *d*STORM[61]. Finally, we demonstrated the versatility of this technique by applying it *in cellulo* to visualize diverse cellular structures, including the microtubule and actin networks, as well as mitochondria in human cells.

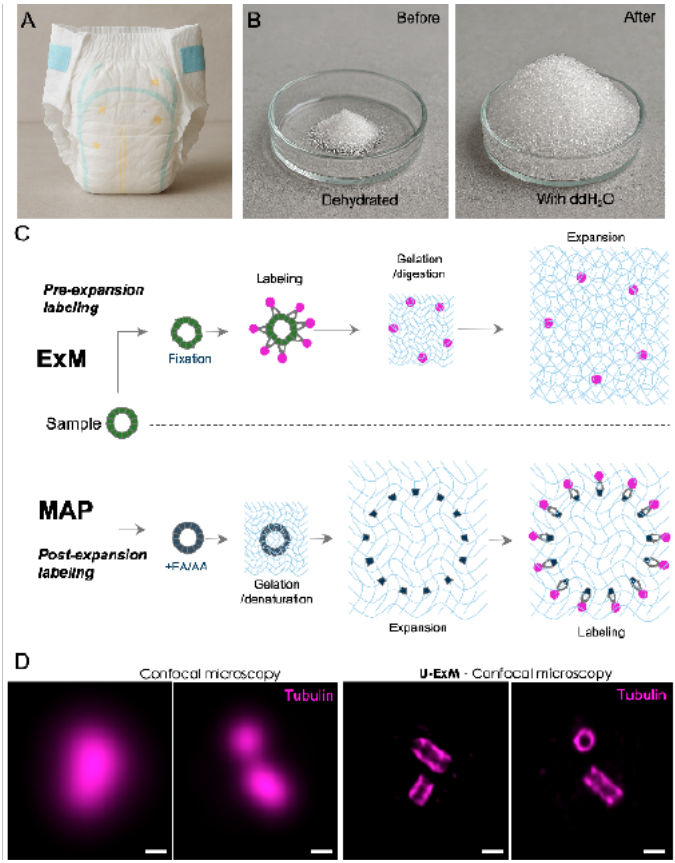

Figure 4: Principle of Expansion Microscopy and post-expansion labeling: The basis of the U-ExM technique. *(A) Baby diaper illustrating the type of source where sodium acrylate powder is used. (B) Absorption property of sodium acrylate, which leads to an increase in volume when pure water is added. (C) Pipeline of the two major ExM/MAP protocols. After fixation biological samples are processed for immunolabeling (ExM) or directly anchored to the polyacrylamide hydrogen owing to formaldehyde and acrylamide anchors (MAP). The proteome of the cell is either digested with an enzyme (proteinase K-ExM protocol) or denatured after gelation, by heating at 95 °C (MAP protocol). After expansion the fluorophore imprint can be imaged (ExM) and labeled with antibodies (MAP). (D) Examples of human centrosome imaged before and after expansion using U-ExM. Scale bar: 200 nm. From Gambarrotto et al, Nature Methods 2019*[61].

Despite the significant advances offered by expansion microscopy, this technique, like all forms of microscopy, is not immune to artifacts. One major source of such artifacts arises during the sample fixation process that precedes expansion. Fixation, while essential for preserving cellular structures, can induce chemical or physical alterations, including protein crosslinking, shrinkage, or redistribution of biomolecules[62, 63]. These changes may distort the native organization of the sample, potentially compromising the spatial accuracy that expansion microscopy seeks to enhance. Therefore, careful optimization and validation of fixation protocols remain crucial to minimizing these artifacts and ensuring reliable interpretation of nanoscale structures.

To tackle this issue, our laboratory developed cryo-expansion microscopy (Cryo-ExM), a technique that integrates the advantages of cryo-fixation, used in the cryo-EM, with those of ultrastructure expansion microscopy[64] ***(Figure 5).*** Cryo-fixation overcomes limitations associated with conventional chemical fixation, which can alter the native cellular state and restrict the range of structures that can be accurately co-analyzed. In this method, biological samples are cryo-fixed via plunge freezing in liquid ethane, resulting in the formation of vitreous ice. The samples are then subjected to freeze substitution, which gradually raises the temperature from −180 °C to 0 °C while replacing intracellular water with acetone, thereby preserving cellular architecture with high fidelity. Following rehydration, the samples are processed using the ultrastructure expansion microscopy (U-ExM) protocol. We demonstrated that Cryo-ExM preserves native cellular organization and allows visualizing several structures of interest such as microtubule, actin networks and membranes simultaneously with best preservation ***(Figure 5A–C).*** We further found that Cryo-ExM improves epitope accessibility and works on a variety of samples and organelles[64] ***(Figure 5D–J).*** Importantly, we also demonstrate that cryo-fixation can also be achieved using high-pressure freezing (HPF) for thicker samples or more sensitive to chemical fixation such as yeast, followed by the cryo-ExM protocol[65].

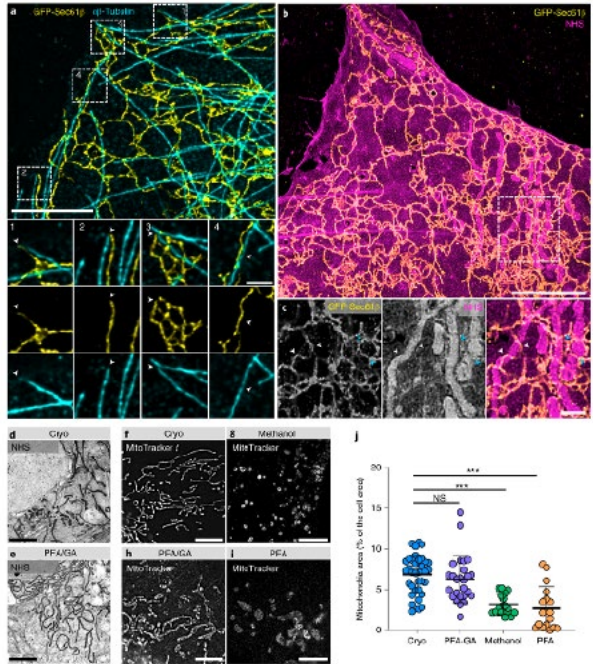

Figure 5: Cryo-ExM. *(A) Confocal image of cryofixed, expanded U2OS cell expressing the ER marker GFP-Sec61β stained with α/β-tubulin (cyan) and GFP (yellow). White dashed squares indicate the position of insets (1–4) showing the ER–MT contacts (white arrowheads). Scale bars, 2.5 µm, 500 nm (insets). (B) Confocal image of cryofixed, expanded U2OS cell expressing the ER marker GFP-Sec61β stained for GFP (yellow) and NHS-ester (magenta). Scale bar, 5 µm (C). Inset from the region depicted by a white dashed square shown in b. ER-mediated wrapping and entanglement of mitochondria are indicated by white and blue arrowheads respectively. Scale bar, 1 µm. (D, E), Confocal images of cryofixed (d) and PFA/GA-fixed (e) U2OS cells, expanded and stained with NHS-ester (NHS, gray). Scale bar, 5 µm. (F-I) Widefield images of expanded U2OS cells incubated with MitoTracker to stain the mitochondrial matrix after cryo (f), methanol (g), PFA/GA (h) or PFA (i) fixations. Scale bar, 5 µm. (J) Quantification of mitochondrial area (% of the cell area) in cryofixed (blue dots), PFA/GA (purple dots), methanol (green dots), PFA (orange dots) cells after expansion. Mean ± s.d., cryo, 6.9 ± 2.4%; PFA/GA, 6.3 ± 2.8%; methanol, 3.2 ± 1.3; PFA, 2.9 ± 2.7%. n = 35, 26, 19 and 17 for cryo, PFA/GA, methanol and PFA, respectively (cryo versus PFA/GA P > 0.999; cryo versus methanol P < 0.0001; cryo versus PFA P < 0.0001; one-way-analysis of variance (ANOVA) followed by Kruskal–Wallis test). NS, not significant. From Laporte et al, Nature Methods 2022*[64].

These two U-ExM-based methods enable a fourfold expansion of biological samples. To further enhance resolution, they can be combined with other super-resolution imaging techniques, as previously discussed, or by increasing the expansion factor itself. The latter strategy has been pursued by several laboratories, including that of Ed Boyden, who first developed the iterative expansion microscopy (iExM) protocol, based on multiple rounds of physical expansion[66]. Another example is Pan-ExM, which combines iterative expansion with pan-cellular labeling using NHS-ester compounds[67]. This pan-labeling allows to label all or most of the proteome of a cell nonspecifically. Unlike conventional labeling, which targets specific proteins or structures (e.g., via antibodies), pan-labeling enables a global visualization of cellular architecture, revealing the overall ultrastructure of the cell or tissue. These approaches have achieved expansion factors ranging from 13x to 21x.

Our laboratory contributed to this field by developing the iterative Ultrastructure Expansion Microscopy (iU-ExM) protocol, which enables expansion factors of 16x to 25x while preserving ultrastructural details at the nanoscale[68] ***(Figure 6).*** iU-ExM builds upon the foundational Ultrastructure Expansion Microscopy (U-ExM) protocol and introduces multiple rounds of gel embedding and expansion to push the resolution further. To achieve a sufficiently strong fluorescent signal, we implemented an intermediate labeling strategy. Using iU-ExM, we successfully revealed the eight-fold symmetry of Nuclear Pore Complexes, macromolecular gatekeepers of the cell nucleus, a structural detail previously accessible only through SMLM or electron microscopy techniques ***(Figure 6A–C).*** We further demonstrate that this method is compatible with cultured cells, whole organisms, and tissues, enabling visualization of molecular periodicities in structures such as in the conoid of the parasite *Toxoplasma gondii* ***(Figure 6D–G)*** and murine photoreceptor cells.

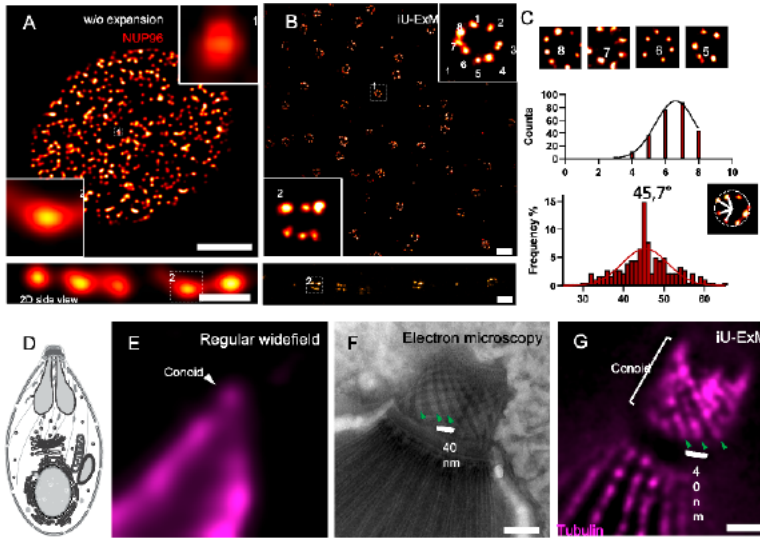

Figure 6: Iterative Ultrastructure Expansion Microscopy (iU-ExM). *(A, B) Upper panel: 2D top view, lower: 2D side view. Confocal image of NUP96-eGFP positive nucleus (red hot) without expansion. Scale bar: 4 µm (upper image), 500 nm (lower), 100 nm (inset). iU-ExM widefield picture of NPCs stained with α-GFP and α-NUP96 in top view (upper image) and side view (lower image). Scales bar: 200 nm (upper image), 50 nm (inset), all corrected. (c) Quantification of the corners per NPCs. N = 202 NPCs from 3 independent experiments. Black continuous line: gaussian regression ($R^2 = 0.96$). (D) Schematic representation of a Toxoplasma gondii parasite. (E) Widefield image of non-expanded T. gondii tachyzoites labelled with tubulin antibodies (magenta) highlighting the conoid region of one parasite (white arrowhead). Scale bar: 1 µm. (F) Electron microscopy of a conoid showing the tubulin fibers (green arrowheads). Scale bar: 100 nm. (G) iU-ExM widefield image unveils the spiral shape of the tubulin fibers of the conoid (green arrowheads). Scale bar: 100 nm corrected. From Louvel et al, Nature Communications 2023*[68].

Overall, the U-ExM-based approaches presented here open new avenues for nanoscale imaging in cell biology, neurobiology, and structural cell analysis, without the need for specialized super-resolution microscopes.

4. Monitoring centriole biogenesis: From nanoscale mapping to function

As we demonstrated that expansion microscopy methods and in particular U-ExM is amenable to unveil the precise localization of proteins within the centriole, we initiated a nanoscale mapping of proteins in human cells. We first identified four proteins localizing at the level of the inner scaffold structure, in osteosarcoma U2OS and RPE1 cells. These proteins are POC1B, FAM161A, POC5, and Centrin ***(Figure 7)***[37]. Moreover, we also identified that these four proteins are part of the same complex, with FAM161A interacting with most of them as well as with microtubules. Additionally, we further identified the human microtubule-binding protein WDR90, whose *Chlamydomonas* homolog POC16 has been proposed to be located at the inner junction of the microtubule triplets at centrioles, as localizing to the inner scaffold region[69] ***(Figure 7A–C).***

Crucially, the identification of the molecular components constituting the inner scaffold of the centriole allowed us to directly interrogate their functional roles. Indeed, U-ExM is a powerful tool for analyzing complex phenotypes, as it greatly enhances the visualization of cellular structures such as centrioles. This improved resolution makes it possible to reveal structural defects that would otherwise remain undetectable. While electron microscopy could also achieve this level of detail, it is a slow, labor-intensive method and lacks the quantitative capabilities offered by U-ExM. Using this approach, we found that targeted depletion of either WDR90 or the central core protein POC5 via siRNA, revealed their essential contribution to maintaining centriole integrity. Specifically, loss of these components resulted in centriolar fracture and disrupted the cohesion between the microtubule triplets that form the centriole wall, a phenotype that aligns with structural predictions derived from high-resolution cryo-electron microscopy data[37]. These findings, further supported by functional assays, provide compelling evidence that WDR90 and POC5 are integral to the mechanical stability of the centriole, reinforcing the proposed model in which the inner scaffold serves as a structural brace that links and stabilizes microtubule triplets ***(Figure 7D)***[69].

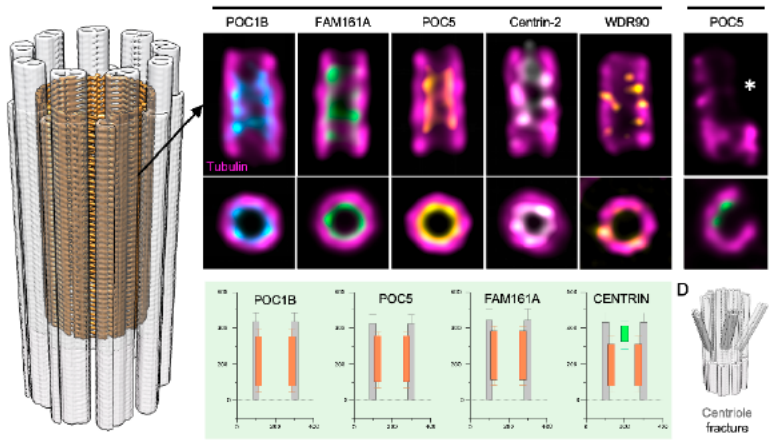

Figure 7: Localization of inner scaffold components and function of inner scaffold components. *(A) 3D model of a centriole highlighting the inner scaffold in yellow/orange. (B) Localization of FAM161A (green), Centrin (white), POC1B (cyan), POC5 (yellow) and WDR90 (orange) revealed using U-ExM; Tubulin is in magenta. Below are the quantifications of the localizations. Adapted from Le Guennec et al, Science Advances, 2020*[37]*. (C) Centriolar structure defects upon removal of WDR90 results in the destabilisation of POC5 as well as its interacting partners. (D) Model of the centriole defect, called centriole fracture, in microtubule triplets/doublets integrity. (C & D) are from Steib et al 2020*[70]*.*

To build on the innovative U-ExM approach that enabled us to identify components of the inner scaffold, we next applied U-ExM to map 24 centriolar proteins with high spatial precision, determining their radial and longitudinal positions to define the molecular nature of distinct structural elements of the centriole[71]. Moreover, by analyzing thousands of centrioles at successive duplication stages, we established a time-series that allowed us to reconstruct the stepwise progression of centriole assembly. The 24 proteins were selected based on prior literature and the availability of antibodies compatible with U-ExM, and included HsSAS-6, STIL, CPAP, CEP135, CEP44, CEP63, CEP152, γ-Tubulin, CCDC77, WDR67, CCDC14, SPICE, CEP295, Centrin, POC5, FAM161A, POC1B, CEP164, C2CD3, CP110, CEP162, and CEP290. Their localization was systematically assessed relative to the tubulin signal that marks the microtubule wall of the centriole ***(Figure 8A–B).***

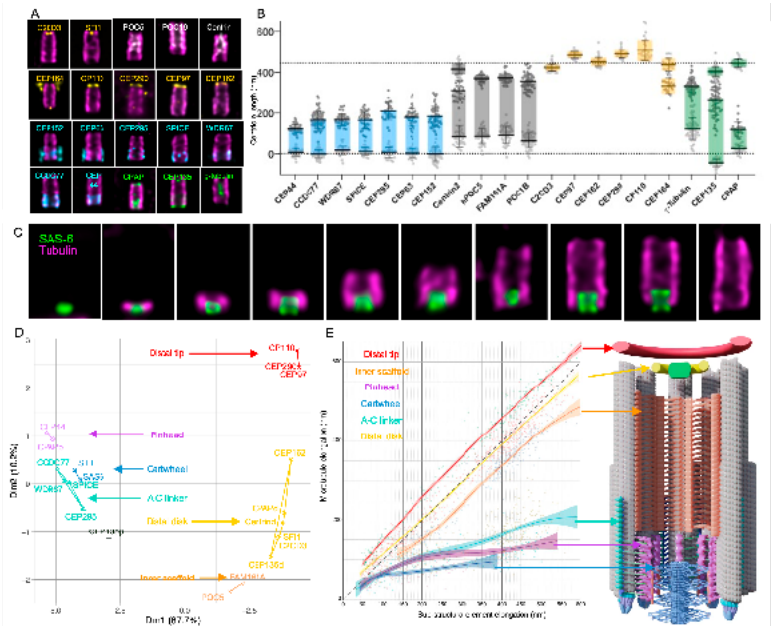

Figure 8: Nanoscale mapping of centriolar proteins using U-ExM to unveil centriole biogenesis: *From Laporte et al. 2024*[71]*. (A) Localization of centriolar proteins along the proximal–distal axis of centrioles. Representative super-resolution images show distinct positioning of proteins involved in cartwheel, linker, scaffold, and distal structures (proteins color-coded, tubulin is in magenta). (B) Quantification of the positions and lengths across proteins (box-and-whisker plots) reveals stratified localization of sub-structural modules. (C) Representative time-series reconstruction of centrioles immunolabeled for SAS-6 (green) and tubulin (magenta), highlighting cartwheel and microtubule triplet growth. (D) Principal component analysis (PCA) of protein localizations groups factors into functional modules including cartwheel, pinhead, A-C linker, distal disk, and distal tip. (E) Sub-structural elongation profiles of centriole modules plotted against overall centriole length. Right: schematic model of centriole architecture integrating quantitative mapping of sub-structural modules.*

Using this dataset, we reconstructed the temporal sequence of human centriole assembly to obtain a dynamic view of its molecular architecture. We found that biogenesis begins with the formation of a «naked cartwheel,» which serves as a scaffold prior to microtubule blade incorporation ***(Figure 8C).*** This is followed by a «bloom phase,» characterized by rapid cartwheel expansion, sequential formation of A-, B-, and C-tubules, and radial separation of structural components. An elongation phase ensues, during which the tubulin backbone extends linearly, the A-C linker is recruited, and inner scaffold proteins are integrated. Our analysis revealed six structural modules that coordinate centriole assembly: the cartwheel, pinhead, A-C linker, inner scaffold, luminal distal ring, and distal cap ***(Figure 8D–E).*** These modules displayed distinct assembly dynamics, with some growing in nonlinear bursts and others in a more gradual manner. Distal proteins emerged early alongside microtubule blades and subsequently capped the centriole tip during elongation. Furthermore, by quantifying large numbers of procentrioles, we detected asymmetries linked to the identity of the parent centriole, which enabled more precise staging of the assembly process[71].

Altogether, our results provide a four-dimensional view of centriole construction, revealing both the molecular identities of key proteins and the dynamic changes in their spatial organization. This framework advances our understanding of how centrioles self-assemble and offers new insight into how disruptions in this process may contribute to disease.

Capitalizing on this groundbreaking work, we next aimed at identifying proteins constituting the A-C linker structure. Since its original description in 1960, no clear protein has been unambiguously attributed to this structural element. Through the localization assessment of the 24 proteins using U-ExM and their classification according to their localization to the proximal, central or distal portion of the centriole, we found a set of 5 proteins, CEP295, SPICE, WDR67, CCDC77 and CCDC14, that display a localization pattern that would be expected from an A-C linker component[71]. From these 5 proteins, we recently focused on two potential uncharacterized A-C linker candidates CCDC77 and WDR67. We demonstrated using U-ExM and iU-ExM that CCDC77, WDR67 and an additional protein identified through a cross DepMap analysis, MIIP,

form a complex and are *bona fide* component of the A-C linker[72]. We then use U-ExM to interrogate the phenotype of depleting A-C linker components. As predicted from electron microscopy data, we found that removal of A-C linker proteins destabilizes centrioles, resulting in structural defects characterized by broken centrioles and the loss of microtubule triplets in the proximal region. Co-depletion of A-C linker proteins and inner scaffold components exacerbated these defects, leading to a higher incidence of centriole breakage. These findings indicate that both structural elements contribute to centriole stability, functioning in distinct yet complementary regions, the proximal and central domains. In addition, we uncovered an unexpected role for A-C linker structure in regulating centriole duplication. This function appears to be mediated by its influence on the recruitment of key proteins, including CEP63 and CEP152, which assemble into a torus structure around the mother centriole and serve as a platform for initiating centriole biogenesis.

5. From centrioles to human pathologies: Retinitis Pigmentosa as a case study

Following the identification of four proteins, FAM161A, POC5, POC1B, and Centrin, that localize to the inner scaffold of centrioles and are associated with the cohesion of microtubule triplets[37, 69], we noticed that some of these proteins were also present at the connecting cilium of photoreceptor cells[73–75]. Intriguingly, mutations of these proteins have been linked to photoreceptor degeneration in previous studies[76–79]. This correlation prompted us to explore the potential connection between our findings on centriole structure and their possible medical relevance in retinal disorders.

In particular, Retinitis Pigmentosa (RP) is a severe inherited retinal disorder, affecting approximately 1 in 4000 individuals worldwide. It leads to progressive vision loss and ultimately results in blindness. Among the various forms of RP, RP28, a ciliopathy uniquely affecting the eye, has been linked to autosomal recessive mutations in the FAM161A gene across multiple ethnic populations[73, 77, 78, 80–82]. In FAM161A-deficient patients, night blindness and visual field constriction typically appear early, during the second to third decade of life[83]. In contrast, visual acuity tends

to decline more slowly, often remaining relatively stable until the fifth or sixth decade, followed by rapid deterioration. This prolonged preservation of central vision offers a broad therapeutic window, spanning three to four decades, for gene augmentation therapies.

To date, no treatments exist that can prevent or restore vision loss in RP28, largely due to a limited understanding of the molecular mechanisms underlying photoreceptor degeneration. To address this, our laboratory employed ultrastructure expansion microscopy (U-ExM) to investigate FAM161A-associated retinal degeneration, aiming to lay the foundation for gene-based therapeutic strategies. We optimized U-ExM for mouse retinal tissue, enabling nanoscale visualization of FAM161A and its interacting partners within photoreceptors ***(Figure 9)***[84].

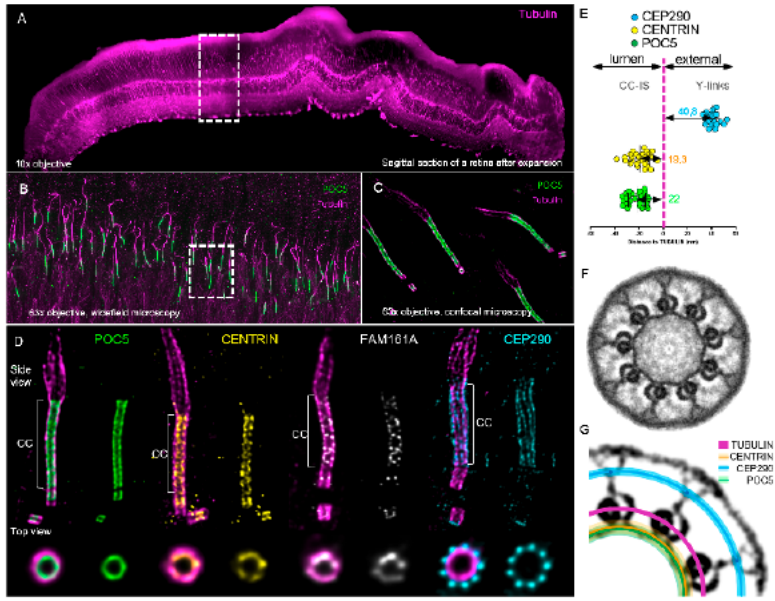

Figure 9: Unveiling the molecular organization of the mammalian photoreceptor connecting cilium. *(A) Low magnification view (10X) of a flat mount retina expanded using U-ExM and stained for tubulin (magenta). (B) Widefield image of an expanded P14 photoreceptor layer stained for tubulin (magenta) and POC5 (green), marking the connecting cilium. (C) Confocal image of a similar region as in B (spotted white box) zooming on few photoreceptor cells. Note that centrioles are visible below the connecting cilium. (D) Confocal U-ExM images of adult photoreceptors stained for tubulin (magenta) and POC5 (green) or CENTRIN (yellow), FAM161A (gray), and CEP290 (cyan). Lower panels show transversal views of the CC for each staining. (E) Distances between the maximum intensity of POC5 (green), CENTRIN (dark yellow), and CEP290 (cyan) compared to tubulin (magenta) calculated from transversal view images. (F) Symmetrized EM image of a P14 CC transversal section revealing an inner ring decorating MTDs and Y-links bridging MTDs to the membrane (G) Model representing relative positions calculated in (E) and (F) of POC5 (green line), CENTRIN (dark yellow line), CEP290 (cyan dot and line) to tubulin (magenta) on a contrasted symmetrized EM picture of a CC. Light color lines represent the SD for each protein. From Mercey et al, PLOS Biology 2022*[84].

Using this approach, we tracked the formation of the connecting cilium during normal mouse retinal development (from postnatal day 4 to 30) and identified a previously uncharacterized structural feature, that we dubbed connecting cilium inner scaffold ***(Figure 10).*** This scaffold, resembling a zipper, is composed of FAM161A and its partners and lines the microtubule wall of the connecting cilium. Importantly, we demonstrated that this scaffold is essential for maintaining microtubule cohesion and axoneme integrity at the connecting cilium. In the FAM161A$^{m1b/tm1b}$ mouse model, which lacks functional FAM161A, the inner scaffold Froms correctly only up to postnatal day 10 ***(Figure 10).*** Thereafter, it disintegrates, leading to microtubule disorganization, collapse of the photoreceptor outer segments, and subsequent cell death[84] ***(Figure 10).***

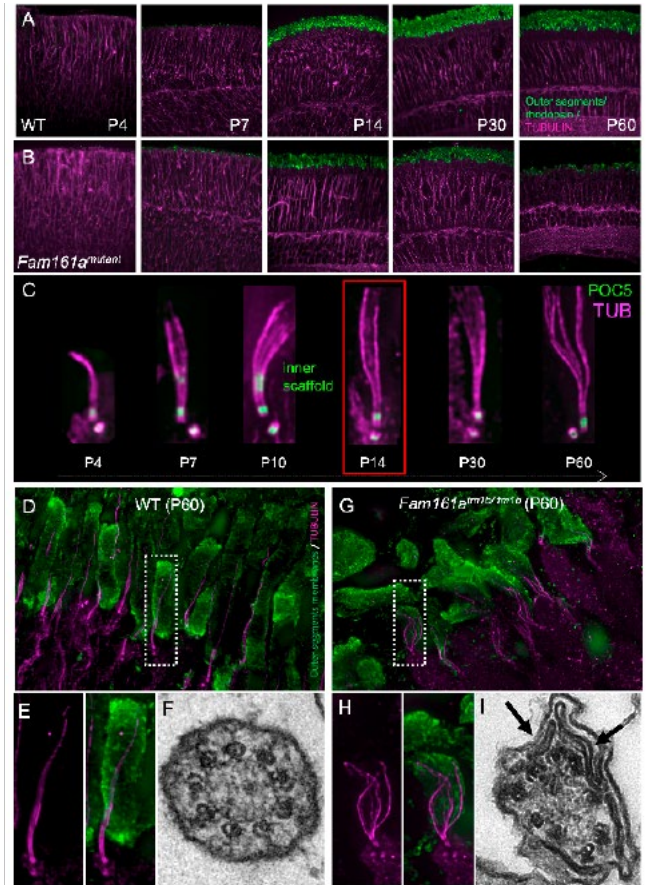

Figure 10: The CC inner scaffold acts as a structural zipper maintaining MTDs cohesion. *(A, B) Low magnification of expanded wild-type (A) or Fam161a$^{tm1b/tm1b}$ (B) retinas showing rod OS formation from P4 to P60 stained with RHODOPSIN (green) and tubulin (magenta). (C) Expanded Fam161a$^{tm1b/tm1b}$ photoreceptors stained for tubulin (magenta) and POC5 (green) from P4 to P60. Note the transitory appearance of the CC inner scaffold between P7 and P10 followed by its collapse, paralleling microtubule spread. (D, G) Low magnification of expanded WT (D) or Fam161a$^{tm1b/tm1b}$ (G) retinas stained for RHODOPSIN (green) and tubulin (magenta) at P60. (E, H) Insets from WT (D) or Fam161a$^{tm1b/tm1b}$ (G) retinas depicted with the white dotted boxes, respectively. (F, I) EM micrographs of WT (F) or Fam161a$^{tm1b/tm1b}$ (I) CC transversal sections. Form Mercey et al, PLOS biology 2022*[84].

We next explored gene therapy as a novel therapeutic strategy for RP28, aiming either to prevent photoreceptor degeneration and vision loss or to restore retinal function. In collaboration with Corinne Kostic and Yvan Arsenijevic (Hospital Jules Gonin, Unil, Lausanne), we evaluated the effects of subretinal injection of various adeno-associated virus (AAV2/8) vectors encoding the human short or long isoforms of FAM161A, driven by different promoters ***(Figure 11).*** Initial experiments using the human IRBP-GRK1 promoter led to ectopic expression of FAM161A throughout the photoreceptor cell body. To better mimic endogenous expression patterns, novel promoters were engineered from regulatory regions of the human FAM161A gene. To more precisely characterize gene therapy outcomes, we optimized the use of U-ExM on cryosections, replacing whole-mount retina preparations ***(Figure 11A–D).*** This approach enabled us to monitor the subcellular localization of FAM161A expression and fine-tune the injection protocol. Notably, a mixture of hFAM161A long and short isoforms resulted in promising preservation of retinal activity three months post-injection[85] ***(Figure 11E).*** This work highlights the essential role of U-ExM in assessing key parameters, such as regulated gene expression, vector dosing, and isoform selection, that are critical for the development of safe and effective gene therapies targeting structural retinal proteins like FAM161A.

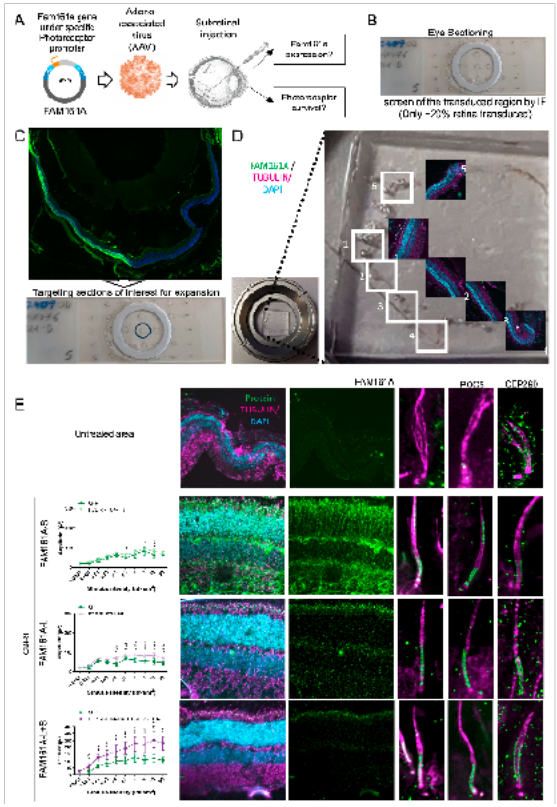

Figure 11: Fine-tuning the efficacy of gene therapy 3 months post injection using U-ExM. *(A) Experimental strategy based on AAV gene therapy for RP28. (B) Image illustrating the cryo-section obtained after eye sectioning to screen for the transduced region. (C) Immunofluorescence showing the positive transduced region of the retina re-expressing FAM161A (green). Blue: DNA. The positive regions in a given cryo-section were proceeded for U-ExM. (D) Expanded cryo-section in an hydrogel deposited in an imaging chamber. (E) Monitoring the re-expression and localization of FAM161A and its partners within the retina and more specifically at the connecting cilium of mouse photoreceptor cells. Tubulin: magenta, green: FAM161A or POC5 or CEP290. (left) Retinal activity measured in the different experimental conditions. Note that solely the injection of FAM161A short and long isoforms rescued visual acuity to some levels and led to the correction localization of FAM161A and its partners in photoreceptor cells and retinal layers. From Arsenijevic et al, EMBO Molecular Medecine 2024*[85].

Together, these findings underscore the critical link between centriole architecture and retinal health, illustrating how deep molecular insights, made possible by advanced techniques like ultrastructure expansion microscopy, can drive the development of targeted gene therapies with the potential to transform the treatment of inherited retinal diseases such as Retinitis pigmentosa.

6. Imaging and immunotherapy: The promise of U-ExM

In immune cells such as cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells, the centrosome undergoes dynamic repositioning during the formation of the immune synapse, a highly specialized and organized interface that forms between an immune effector cell and its target. This centrosome polarization is a crucial step that brings the microtubule-organizing center (MTOC) into close proximity with the synaptic membrane, thereby facilitating the directional trafficking and focused secretion of cytotoxic granules toward the target cell. Such precise intracellular reorganization ensures the efficient elimination of virally infected or malignant cells while minimizing damage to surrounding healthy tissue. Gaining a deeper understanding of the molecular and structural changes that occur during immune synapse formation is therefore essential, not only for advancing basic immunological knowledge but also for informing clinical strategies, particularly in the context of engineered cell therapies such as chimeric antigen receptor (CAR) T cells, where optimizing synapse formation and granule delivery could significantly enhance therapeutic efficacy.

To tackle this question, we turned to cryo-ExM, which best preserve cell architecture [64]. We first demonstrated that cryo-ExM effectively preserves the ultrastructural features of the immunological synapse in both Jurkat T cells and primary T cells derived from healthy donors, activated on functionalized surfaces to mimic synapse formation[86]. To confirm isotropic expansion, we quantitatively compared the nuclear area in both expanded and non-expanded conditions, demonstrating a consistent ~4-fold enlargement across samples. Cryo-ExM provided exceptional spatial resolution, enabling detailed visualization of key cytoskeletal elements, including actin and microtubule networks (***Figure 12).*** Notably, our anal-

ysis revealed that the centrioles within T cells are significantly shorter than those observed in canonical centrosomal models, such as U2OS cells. This structural deviation may have functional implications for immune synapse assembly, particularly in the context of centrosome docking at the plasma membrane, a critical step in polarized secretion during T cell activation[86].

Importantly, cryo-ExM enabled the direct imaging of immunological synapse formation and associated cytoskeletal remodeling in both Jurkat and primary T cells using standard epifluorescence microscopy. This level of structural detail, previously accessible only through advanced super-resolution approaches, demonstrates the power of cryo-ExM to bridge the resolution gap in routine imaging workflows. Moreover, the technique uncovered fine cellular features, such as tunneling nanotubes (TNTs), thin membranous channels connecting cancer cells[87] These structures, implicated in the intercellular transfer of organelles, signaling molecules, and even cytotoxic agents, may play a significant role in tumor progression and immune evasion. The ability to resolve such delicate architectures underscores cryo-ExM's potential for advancing our understanding of dynamic cell-cell communication in immunological and oncological contexts.

With a robust experimental protocol in place, our subsequent objective was to visualize synaptic molecules implicated in T cell response, with a particular focus on the cytotoxic machinery involved in target cell killing. To achieve this, we tracked the localization of key effector components, such as Granzyme B, Perforin, and LAMP1. In parallel, we successfully visualized mitochondria using the TOMM20 marker and labeled synaptic membrane lipids with the mCLING probe (REF). These results underscore the ability of cryo-ExM to preserve and reveal intricate cellular architecture with high fidelity.

Crucially, our approach provides new insights into the spatial organization and deployment of the cytotoxic apparatus during T cell-tumor cell interactions. Utilizing validated markers such as LAMP1, Perforin, and Granzyme B as mentioned ***(Figure 12A, E),*** we could resolve individual LAMP1-positive vesicles. Remarkably, we observed that some vesicles

co-contained both Granzyme B and Perforin, enzymes responsible for inducing apoptosis and membrane lysis, respectively, and that these vesicles clustered in proximity to the immunological synapse.

For the first time, cryo-ExM allowed us to distinguish between different types of cytotoxic granules in T cells, specifically the recently described multi-core granules (MCG) and single-core granules (SCG) ***(Figure 12B).*** Previously, these structures were only observable via electron microscopy[88]. Our imaging confirms that LAMP1-positive granules vary in content and structure, and that their size and abundance align with previous findings[88, 89].

Furthermore, we successfully expanded and imaged T cell–tumor cell pairs using cryo-ExM, achieving sufficient resolution to visualize the immunological cleft, a narrow 10–50 nm interface traditionally accessible only through electron microscopy or advanced super-resolution techniques ***(Figure 12 C–E).*** This capability highlights cryo-ExM as a powerful tool for dissecting the nanoscale architecture of immune synapses and the dynamics of T cell cytotoxicity.

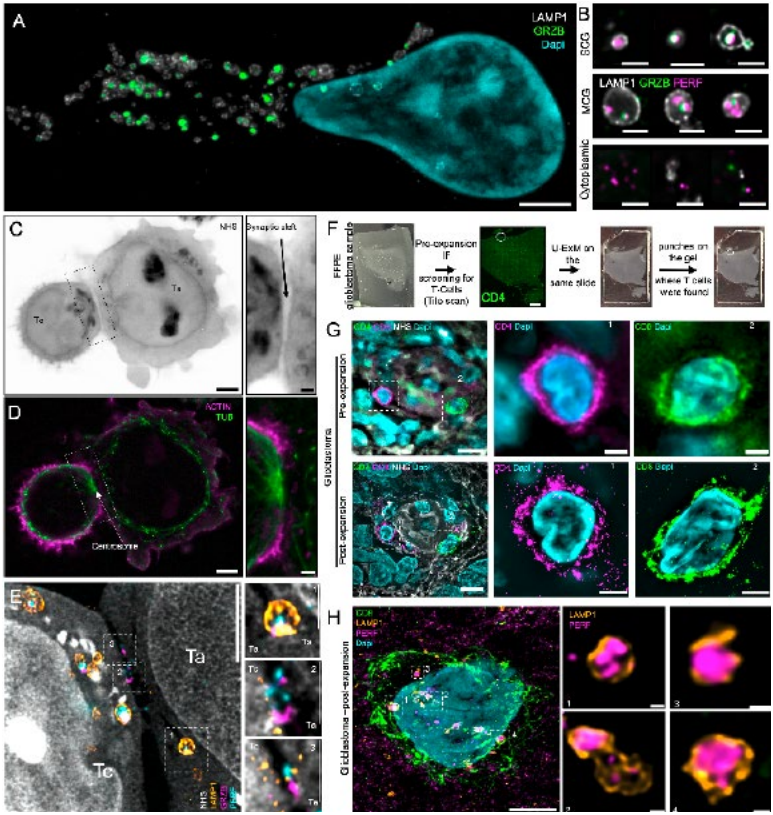

Figure 12: Immune synapse and its killing machinery revealed by Cryo-ExM. *(A) Widefield image of cryo-fixed, expanded primary T cell labelled for LAMP1 (grey), Granzyme B (GRZB, green) and nucleus (DAPI, cyan). Scale bar: 2 µm. (B) Widefield images of CD8 T cell single core (SCG), multicore (MCG), and cytoplasmic lytic granules containing one or multiple GRZB (green) and PERF (magenta) foci surrounded by LAMP1 (grey) or not (cytoplasmic), scale bar: 500 nm. (C, D) Widefield fluorescent images of cryo-fixed, expanded pairs of engineered TCR-transgenic (HLA-A2 survivin) human T cells (Tc) and BV173 target (Ta) cells labelled for NHS (inverted greyscale, C), actin (magenta, D) and tubulin (green, D). Insets magnify the synaptic cleft. Note in (D) the centrosome polarization and actin exclusion from the synapse interface, scale 2µm. (E) Confocal images of a cryo-fixed and expanded TCR-engineered (anti-survivin) human T cell (Tc) and BV173 target cell (Ta) pair labelled for NHS (grey), LAMP1 (orange), Granzyme B (magenta) and Perforin (cyan).*

Insets one, two and three magnify LAMP1 vesicle containing Granzyme B and Perforin in the target cell (inset 1), and free Granzyme B and Perforin within the synaptic cleft (inset 2 and 3), left scale bar: 2 µm, inset scale bar: 500 nm. (F) Workflow for expanding FFPE glioblastoma sections. Non-expanded tissue sections are labeled for CD4 or CD8 maker and a tile scan of the entire tissue is performed using widefield fluorescent microscope (10x objective). The tissue is then screened with a 63x objective for regions of interest. A picture is subsequently taken using a 10x objective to map the regions of interest before performing U-ExM procedure on the tissue sample. Gel punches are then made to isolate the previously identified region of interest and stained for CD4, CD8 and proteins of interest. After imaging, an additional stripping step can be added for multiplexing. (G) Widefield fluorescent image of a pre-expansion (upper row) FFPE section of human glioblastoma tissue stained for CD4 (green), CD8 (magenta), nucleus (DAPI, cyan) and NHS (gray) and post-expansion (lower row) of the same sample that was re-labeled for the same cell markers. The same field of view is presented for the merge channel image and the insets 1 and 2. Individual CD4 and 746 CD8 T cells can be detected, scale bar: 2 µm. (H) Confocal image of expanded FFPE glioblastoma section labeled for CD8 (green), LAMP1 (orange), Perforin (magenta) and nucleus (Dapi, cyan). Insets 1–4 show single core and multicore granules within a CD8 T cell, left scale bar: 2 µm, inset scale bar: 100 nm. From Lemaitre et al, BioRxiv 2025[86].

Next, we set out to explore the potential of expansion microscopy for clinical applications. To achieve our goals, we implemented U-ExM on human cryo-fixed or formalin-fixed paraffin-embedded (FFPE) human tissue specimens from the HUG Pathology Department (Geneva) ***(Figure 12F–H).*** The long-term goal of this effort is to investigate the *in situ* interactions between T cells and tumor cells, particularly in the context of glioblastoma, in collaboration with Prof. D. Migliorini (ISREC, HUG, and UNIGE). We demonstrate that we can expand and preserve both cryo-fixed and FFPE sections from different human healthy or diseased tissues ***(Figure 12F–H)***[86]. This approach could reveal extremely powerful to understand what is happening *in situ* and could be applied in the long-term in patient's biopsies.

7. Perspectives: Unlocking the molecular landscape of disease

The story of biology has always been shaped by new ways of looking at life. Expansion microscopy continues this tradition, but with a twist: instead of building ever more complex microscopes, it physically enlarges the sample itself, allowing ordinary instruments to reveal extraordinary

detail. What began as a clever physical trick is now emerging as a method that can genuinely transform biomedical research and, in the long run, clinical practice.

For biomedicine, the key contribution of expansion microscopy is that it turns the invisible into the visible. At the nanoscale, small shifts in the organization of proteins, organelles, or membranes can make the difference between health and disease. By gently stretching tissues and cells while preserving their architecture, expansion microscopy opens up these crowded molecular spaces, allowing researchers to see subtle defects in organelles such as centrioles, cilia, or synapses, defects that underlie a wide spectrum of disorders, from cancer to inherited blindness to immune dysfunction.

In fundamental research, this technique has already provided a molecular blueprint of the centriole and helped uncover how its disruption can destabilize entire cells. In the retina, it has revealed a hidden «scaffold» structure whose failure contributes to retinitis pigmentosa, suggesting new ways to evaluate and improve gene therapy. In immunology, it has given us a direct view of how killer T cells and cancer cells confront each other across the narrow space of the immune synapse, with implications for the design of next-generation cell therapies. Each of these examples illustrates the same principle: when we can see biological machinery at its true scale, we understand not only how it works, but also how to intervene when it fails.

While expansion microscopy has undoubtedly reshaped our understanding of human biology and disease, its impact reaches much further. These methods have proven extremely powerful in the study of small organisms, offering levels of spatial resolution that were previously unattainable with conventional imaging. From parasites such as Toxoplasma gondii[90], Plasmodium spp. [91], and Trypanosoma spp.[92], to the intricate cellular architecture of plankton[93], expansion microscopy has opened new avenues for exploring cellular structures and life processes at the nanoscale. By enabling detailed visualization of such diverse systems, it not only advances our understanding of pathogenic mechanisms but also contributes to broader ecological and evolutionary studies. This highlights a remarkable versa-

tility: a single approach that can illuminate both the molecular underpinnings of human disease and the fundamental architecture of life across the tree of organisms.

The promise for medicine is equally profound. Expansion microscopy can be applied not only to research samples but also to human tissue, including the standard paraffin blocks that fill hospital pathology archives[85, 94]. This raises the possibility of revisiting years of patient material with new eyes, uncovering nanoscale features that may serve as powerful biomarkers of disease progression or therapeutic response. In cancer, for example, we might one day stratify patients not only by genetic mutations but also by the nanoscale organization of their tumor cells and their interactions with immune cells. In neurology, subtle changes in synaptic organization could serve as early warnings for degenerative disease. In infectious disease, the architecture of host–pathogen interactions could guide treatment strategies.

Challenges remain, of course. The methods must be made faster, simpler, and more standardized before they can be adopted in clinical settings. Yet one of the strengths of expansion microscopy is its accessibility: because it works with conventional microscopes, its benefits are not confined to a handful of elite centers. With the right protocols, kits, and training, it could become a widely available bridge between cutting-edge nanoscale biology and everyday medical practice.

Looking ahead, we see expansion microscopy as part of a new diagnostic language, complementing genetics and histology. Where sequencing deciphers the code and pathology shows the tissue context, expansion microscopy adds the missing dimension: the nanoscale architecture of disease. By enabling us to see life at the scale where it is built, it holds the promise of more precise diagnoses, better therapeutic monitoring, and, ultimately, more personalized treatments. In short, expansion microscopy invites us to imagine a future in which the fine architecture of cells and tissues is no longer hidden, but becomes a routine part of how we understand, diagnose, and treat human disease. It is a future where seeing truly is healing.

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THE CLOËTTA PRIZE 2025
IS AWARDED TO

PROFESSOR

NICOLA ACETO

BORN IN 1982 IN ALESSANDRIA, ITALY,

FULL PROFESSOR OF MOLECULAR ONCOLOGY AT ETH ZURICH

FOR HIS GROUNDBREAKING RESEARCH ON CANCER METASTASIS, REVEALING THE PIVOTAL ROLE OF CIRCULATING TUMOR CELL CLUSTERS (CTC CLUSTERS) AND DEVELOPING INNOVATIVE THERAPEUTIC STRATEGIES WITH THE POTENTIAL TO IMPROVE TREATMENT OF AGGRESSIVE CANCERS

ZURICH, 31ST OCTOBER 2025

IN THE NAME OF THE FOUNDATION BOARD:

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THE VICE PRESIDENT

A MEMBER

NICOLA ACETO

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Current Position

2024–Present **Full Professor of Molecular Oncology,** Department of Biology, ETH Zurich, and **Vice-Chairman,** Institute of Molecular Health Sciences (IMHS), ETH Zurich

Previous Positions

2021–2024 **Associate Professor (with tenure) of Molecular Oncology,** ETH Zurich

2015–2021 **Group Leader and Swiss National Science Foundation Assistant Professor of Oncology**
Department of Biomedicine, University of Basel, Switzerland

2012–2015 **Research Fellow in Medicine, Harvard Medical School,** Massachusetts General Hospital Cancer Center, Boston, MA, USA (Mentor: Prof. D. A. Haber)

2014–2015 **Associate external member, Broad Institute of MIT and Harvard,** Cambridge, MA, USA

2014 **Visiting Scientist, Hubrecht Institute,** Utrecht, The Netherlands (mentor: Prof. H. Clevers)

Education

14/02/2014	**Cancer Computational Biology Program** Broad Institute of MIT and Harvard, Cambridge, MA, USA
19/05/2011	**PhD in Biochemistry** ***(summa cum laude and best FMI PhD thesis 2011)*** Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland
17/07/2006	**Master's degree in Medical and Pharmaceutical Biotechnology** ***(summa cum** laude)* University «Amedeo Avogadro», Novara, Italy
27/07/2004	**Bachelor degree in Biotechnology** ***(magna cum laude)*** University «Amedeo Avogadro», Novara, Italy

Grants (3rd Party Funding)

2015–Present:	**> CHF 15 500 000 total** This includes: 3x **ERC** grants, 6x **SNSF** grants, 5x **Swiss Cancer League** grants, several other grants from the **European Union, Foundations** and **Funding Agencies.**

Peer-Reviewed Publications

2008–Present:	**> 90 peer-reviewed publications; > 13 500 citations; > 44 h-index**
These include:	Highly cited publications in **Cell; Nature; Science; Nature Medicine; Nature Genetics; Nature Methods; Nature Reviews Cancer.**

Honors

2006–Present: **> 25 awards**

These include: **Precision Medicine Luminary Award,** Silicon Valley, CA, USA (2025); **Dandelion Entrepreneurship Award,** ETH Zurich (2024); **Robert Wenner Award,** Swiss Cancer League, Bern, Switzerland (2023); **The Pezcoller Foundation-EACR Translational Cancer Research Award,** European Association for Cancer Research and Pezcoller Foundation (2023); **Swiss Science Prize Latsis,** Swiss National Science Foundation, Bern, Switzerland (2022); **Friedrich Miescher Award** for Outstanding Achievements in Biochemistry, Life Sciences Switzerland LS^2, Zürich, Switzerland (2020); **Honorable mention,** AAAS Wachtel Cancer Research Award, USA (2016)

Invited Speaker

2009–Present: **> 260 invitations (> 27 keynotes)**

These include: **Distinguished Lecture, German Cancer Research Center (DKFZ),** Heidelberg, Germany (2025); **Cancer Research UK, Manchester Institute,** United Kingdom (2024); **Grand Rounds, Comprehensive Cancer Center Zurich,** Switzerland (2024); **Metastasis Research Society, The Francis Crick Institute**, London, UK (2024); **CNIO Frontiers Meeting on Metastasis,** Madrid, Spain (2023); **The ISREC-SCCL Symposium, The Swiss Institute for Experimental Cancer Research,** Lausanne, Switzerland (2023); **The Weizmann Institute of Science,** Rehovot, Israel (2023); **Plenary Session, AACR Special Conference on Cancer Metastasis,** Portland, OR, USA (2022); **Genentech,** San Francisco, CA, USA (2022); **Keynote lecture, Annual Meeting of the Japanese Association for Metastasis Research,** Kyoto University, Kyoto, Japan (2022); **5th EACR**

Conference on Cancer Genomics, Oxford Brookes University, Oxford, UK (2022); **Pfizer AG,** Switzerland (2022); **Plenary Talk and Session Chair, Keystone Symposia, Precision Oncology: Translating Discovery to the Clinic, Van Andel Institute,** Grand Rapids, MI, USA (2021); **Lola and Grace Distinguished Lecture in Cancer Research, EPFL,** Lausanne, Switzerland (2021); **San Antonio Breast Cancer Symposium,** San Antonio, Texas, USA (2019); **Northwestern University Feinberg School of Medicine,** Chicago, IL, USA (2019); **Cancer Biology Seminar, Memorial Sloan Kettering Cancer Center,** New York City, NY, USA (2019); **Novartis Institute for Biomedical Research,** Oncology Division, Basel, Switzerland (2019); **Gordon Research Conference (GRC) on Mammary Gland Biology,** Lucca, Italy (2018); **7th Annual Circulating Tumor Cell and Liquid Biopsy Meeting,** San Francisco, CA, USA (2017).

Conferences Organization

2017–Present: **> 7 conferences organized**
These include: **European Association for Cancer Research (EACR), Liquid Biopsy,** Lyon, France (2024)

Intellectual Property (Patents Filed and/or Granted, Software Development)

2011–Present: **> 16 patents filed**

Commissions of Trust (Funding Agencies)

2011–Present:	Reviewer for **> 45 Funding Agencies**
These include:	**European Research Council** (ERC); Member of Scientific Committee, **Swiss Cancer League,** Bern, Switzerland; **The Swiss National Science Foundation** (SNSF), Switzerland; Member of Scientific Committee; **Luxembourg National Research Fund** (FNR); **Cancer Research UK** (CRUK); **Medical Research Council** (MRC), UK; **Boehringer Ingelheim Fonds,** Germany; **The Swedish Cancer Society,** Stockholm, Sweden; **The Dutch Cancer Society** (KWF), The Netherlands; **The Canadian Cancer Society** (CSC), Canada; **AIRC Foundation for Cancer Research,** Italy; **French National Research Agency** (ANR), Paris, France.

Commissions of Trust (Scientific Journals)

2021–Present:	Editor for **Cancer Research (AACR)** and **eLife**
2011–Present:	Reviewer for **> 110 scientific journals**
These include:	**Nature; Science; Cell; Nature Medicine; Nature Cell Biology; Nature Reviews Cancer; Cancer Cell; Cell Reports; Cell Stem Cell; Science Translational Medicine; Cancer Discovery.**

Commissions of Trust (Boards and Consultancy Activity)

2016–Present:	Consultant, board member or scientific panel member > 30 academic or private entities
These include:	Member, **Strategy Committee of the Department of Biology, ETH Zurich,** Switzerland; Member, **ETH Tenure Committee, ETH Zurich,** Switzerland; Member, **Scientific Advisory Board**, **Swiss Cancer League** and **Swiss Cancer Research Foundation,** Bern, Switzerland; Co-Founder, Chief Scientific Advisor and Member of the Board of Directors, **PAGE**

Therapeutics AG, Switzerland; Chairman of the Scientific Advisory Board and Consultant, **TETHIS S.p.A.**, Milan, Italy; Review Panel Member, **Luxembourg National Research Fund (FNR);** Ad-hoc Consultant, **Swiss Re,** Zurich, Switzerland.

Teaching

2016–Present:	> 13 courses
These include:	**Concept Course** in Cell Biology of Health and Disease (ETHZ); **Basic Course in Cell Biology,** Molecular Mechanisms of Health and Disease (ETHZ); **Block Course** in Cancer Progression (ETHZ).

Direct Supervision of Employees, Students and Research Associates (Aceto Lab)

2015–Present	> 58 lab members
These include:	**1 senior scientist, 1 lab manager, 1 executive administrative assistant, 1 senior computational biologist, 1 program manager, 8 postdoctoral fellows, 3 clinical fellows, 1 MD-PhD fellow, 17 PhD students, 7 lab technicians, 9 master students, 8 semester students.**
Among these:	> 13 received personal grants/fellowships/awards while in the lab – e.g. Marie Sklodowska-Curie Actions Individual Fellowship; EMBO Fellowship; ETH Pioneer Fellowship.

Memberships of Scientific societies

2007–Present	> 16 scientific societies
These include:	The **European Society for Medical Oncology (ESMO),** Lugano, Switzerland; The **Metastasis Research Society (MRS),** Tampa, FL, USA; The **European Association for Cancer Research (EACR),** Nottingham, UK; The **American Association for Cancer Research (AACR),** USA.

Selected Publications

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BIOLOGY AND THERAPEUTIC TARGETING OF CIRCULATING TUMOR CELL CLUSTERS

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Introduction

Carcinomas, malignancies arising from epithelial tissues, represent the predominant class of human cancers and account for most cancer-related deaths worldwide (1). These tumors emerge from epithelial linings that constitute the surfaces of organs, glands, and mucosal barriers, giving rise to a diverse spectrum of disease entities across nearly every anatomical site. Despite this heterogeneity in presentation, carcinomas are unified by their global prevalence, high incidence, and considerable clinical impact (1). The crucial clinical challenge related to carcinomas lies not in the presence of the primary tumor itself, but in their capacity for dissemination. Localized carcinomas, when detected early, are often amenable to surgical excision or localized therapies and may, in some cases, be curable. In contrast, once tumor cells spread to distant organs, therapeutic options are scarce, and long-term survival is rarely achievable (2). Thus, metastatic progression is the principal driver of morbidity and mortality in carcinoma patients.

Carcinomas exhibit organ-specific patterns of spread, shaped by both intrinsic properties of tumor cells and permissive niches within secondary organs. Some display a predilection for osseous dissemination, while others preferentially colonize the liver, lung, or brain. These tropisms appear not to be random but reflect complex biological compatibilities between disseminated carcinoma cells, circulation dynamics and host tissue microenvironments (3). Clinically, this translates into divergent prognoses across carcinoma types, but the unifying theme remains that metastatic involvement dramatically worsens survival and quality of life.

Globally, millions of patients are diagnosed with carcinoma annually, and a significant fraction will progress to advanced, disseminated disease. Since curative treatments for late-stage carcinoma are limited, the pre-

vention or delay of metastasis represents a critical unmet need in oncology. A rigorous understanding of the processes by which carcinoma cells disseminate is therefore central to advancing therapeutic innovation. This necessity directs attention to the metastatic cascade, the multistep journey through which primary epithelial tumor cells evolve into colonizers of distant organs.

The metastatic cascade

Metastasis is the defining feature of malignant carcinoma progression. It is not a single event but a cascade of interdependent biological steps that together enable cancer cells to leave the primary site, survive systemic transit, and establish secondary growths in foreign tissues (4). The cascade begins with local invasion, whereby carcinoma cells breach the basement membrane and infiltrate the surrounding stroma. Once invasive, carcinoma cells may intravasate into the circulatory system directly through blood vessels or indirectly *via* the lymphatic network. Circulating through the vascular system represents a hostile phase of the metastatic journey. Tumor cells are subjected to hemodynamic shear stress, loss of anchorage, and continuous immune surveillance. As a result, only a minute fraction of circulating carcinoma cells survives long enough to reach distant tissues. Successful extravasation and dissemination into target organs require dynamic interactions with endothelial cells, remodeling of local vascular barriers, and exploitation of tissue-specific molecular cues. Upon dissemination, organ colonization appears to be the most rate-limiting and clinically consequential step of the cascade. Disseminated carcinoma cells must either adapt to new microenvironments or enter dormant states to evade immune clearance and therapeutic elimination. Reactivation from dormancy, followed by proliferative outgrowth, underlies the eventual emergence of clinically detectable metastases. Importantly, this colonization process is influenced not only by tumor cell-intrinsic traits but also by the permissiveness of the «soil,» a concept long captured by Paget's «seed and soil» hypothesis (2).

In recent years, phylogenetic and evolutionary studies have refined our understanding of this cascade. Increasing evidence indicates that metastasis-to-metastasis spread plays a critical role in shaping the natural his-

tory of advanced disease, often surpassing the frequency of direct dissemination from the primary tumor (5). This self-propagating metastatic network creates a moving target for therapeutic intervention and underscores the need for systemic strategies capable of intercepting metastatic progression at multiple stages. From a clinical perspective, this insight has given rise to new paradigms in patient management. The notion that interventions may target dissemination itself – rather than focusing exclusively on established lesions – represents a profound conceptual shift. In particular, patients with limited metastatic burden could benefit from approaches that combine local ablation with systemic therapies designed to suppress further spread. However, identifying and characterizing the cellular mediators of dissemination remains a fundamental prerequisite for developing such strategies. Central to this effort are circulating tumor cells.

Circulating tumor cells

Circulating tumor cells (CTCs) are carcinoma cells that detach from either primary tumors or existing metastatic lesions and enter the bloodstream, either directly or indirectly (e.g. *via* the lymphatics). They occupy a pivotal role in the metastatic cascade as the main intermediaries linking tumor initiation to distant colonization. Importantly, their accessibility through liquid biopsy provides a unique opportunity to study the biology of dissemination in real time. CTCs exist in diverse forms. They may circulate as individual cells or as multicellular aggregates known as CTC clusters. These clusters can be homotypic, consisting entirely of carcinoma cells, or heterotypic, incorporating stromal or immune elements that confer cooperative advantages. Accumulating evidence suggests that CTC clusters exhibit greater metastatic efficiency than solitary cells, due to enhanced physical and biological properties (6).

Clinically, CTC enumeration and characterization have emerged as powerful prognostic tools across multiple carcinoma types. Elevated CTC counts in patient blood samples consistently correlate with poor clinical outcomes, reflecting higher risks of progression and mortality (7–9). Moreover, beyond mere enumeration, molecular profiling of CTCs has revealed insights into treatment resistance, phenotypic plasticity, and

metastatic tropism (6). From a therapeutic standpoint, CTCs present dual opportunities. As biomarkers, they enable dynamic monitoring of disease progression and treatment response with minimally invasive blood sampling. As biological entities, they represent potential targets for interventions designed to prevent systemic dissemination or interrupt cluster-mediated colonization. By targeting CTCs, it may be possible to disrupt the continuity of the metastatic cascade, thereby reducing the likelihood of progression to incurable late-stage disease.

Given the ubiquity of carcinomas and the universality of metastasis as a life-limiting event, the study of CTC biology is of broad relevance across epithelial malignancies. Investigating how CTCs survive systemic circulation, interact with the host microenvironment, and seed distant lesions is likely to enhance our mechanistic understanding of metastasis but also inform the development of novel therapeutic strategies.

Homotypic CTC clusters

CTCs represent the cellular intermediates of the metastatic cascade, bridging primary tumor shedding with colonization of distant organs. While CTCs are most frequently detected as individual epithelial cells, a subset circulate as multicellular aggregates, or clusters. These CTC clusters can be homotypic, consisting exclusively of carcinoma cells, or heterotypic, incorporating stromal or immune elements. Among these, homotypic CTC clusters have attracted particular attention because they provide a striking example of how collective cellular behavior can amplify the metastatic potential of carcinoma. Although relatively rare in circulation – comprising only 2–5% of total CTC events – homotypic clusters disproportionately contribute to metastatic burden due to their markedly elevated competence for colonization (10).

We previously demonstrated that homotypic CTC clusters do not arise from intravascular aggregation of single cells, but rather from oligoclonal groupings of neighboring tumor cells that detach collectively from the primary tumor mass. Using mouse models engineered with fluorescently labeled breast cancer cells, we showed that most clusters contained multiple distinct clones, reflecting their derivation from primary tumor frag-

ments (10). This oligoclonality excludes the possibility that clusters are simply proliferative outgrowths of a single CTC within the vasculature. Mechanistically, clusters are stabilized by intercellular adhesion proteins, with plakoglobin identified as a critical determinant. Transcriptomic profiling revealed plakoglobin as strongly enriched in clustered *versus* single CTCs. Knockdown of plakoglobin disrupted cluster integrity and significantly reduced metastatic seeding, establishing adhesion-mediated cohesion as both a structural and functional requirement for cluster biology (10).

Perhaps the most striking feature of homotypic clusters is their extraordinary metastatic efficiency. In mouse xenograft models, we quantified that clusters exhibit up to 50-fold higher metastatic potential compared with single CTCs ***(Fig. 1)***.

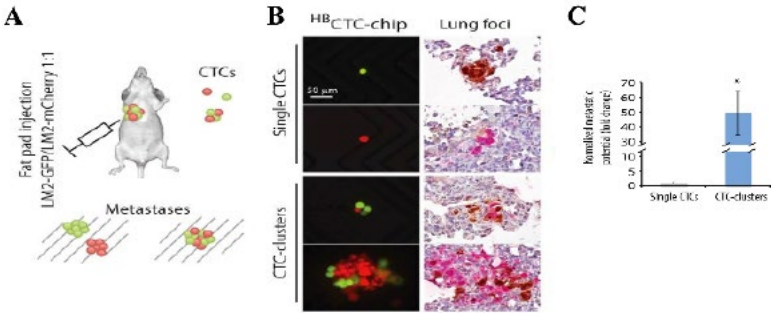

Figure 1*. CTC Clusters Demonstrate Increased Metastatic Potential Compared to Single CTCs.* ***(A)*** *Schematic of the experiment. MDA-MB-231-LM2 (LM2) cells expressing GFP (LM2-GFP) or mCherry (LM2-mCherry) cells are mixed at 1:1 ratio and injected in the right mammary gland of immunodeficient mice to generate one-color single CTCs and multicolor CTC clusters. Accordingly, one-color metastatic foci are derived from a single CTC, while multicolor foci arise predominantly from a CTC cluster.* ***(B)*** *Representative images of single CTCs (GFP- or mCherry-positive) and CTC clusters (GFP- and mCherry-positive) (left). Lung metastatic foci derived from a single CTC (GFP- or mCherry-positive) or a CTC cluster (GFP- and mCherry-positive) are shown (right). GFP (brown), mCherry (red).* ***(C)*** *Bar graph showing the normalized metastatic potential of single CTCs and CTC clusters.*

This heightened metastatic competence derives from multiple physical and biological advantages. First, the preservation of cell–cell junctions within clusters provides structural integrity and a higher likelihood for CTC clusters to be trapped within capillary beds of distant sites, offering an ideal docking tool to be exploited at a distant site. Second, clusters demonstrate enhanced resistance to apoptosis following dissemination into lung tissue, as evidenced by reduced caspase-3 activation compared with single CTCs (10). Third, the oligoclonal composition of clusters, recently demonstrated at the mutational level in patients (11), may foster cooperative interactions between genetically diverse tumor cells, thereby enhancing adaptability in hostile microenvironments. In patients, the clinical significance of clusters was corroborated by our initial observation that their presence in blood samples correlated with poor prognosis in breast and prostate cancer cohorts – a finding that has now been reproduced in a variety of carcinoma types (12, 13). Thus, both experimental and clinical data converge on the conclusion that homotypic clusters represent a rare but disproportionately lethal subset of CTCs.

Building on these observations, we interrogated the epigenetic landscape of clusters to uncover molecular features underlying their metastatic potency. Through single-cell resolution whole-genome bisulfite sequencing, we compared DNA methylation profiles of homotypic CTC clusters and single CTCs from both breast cancer patients and mouse xenograft models (14). The results revealed a distinctive signature: homotypic clusters displayed widespread hypomethylation at transcription factor binding sites associated with pluripotency and self-renewal, including OCT4, NANOG and SOX2. This pattern parallels embryonic stem cell methylomes, suggesting that clustering endows carcinoma cells with epigenetic programs that reinforce stemness, proliferation, and metastatic seeding capacity. In contrast, single CTCs exhibited hypomethylation at TFBSs linked to stress responses and differentiation, but not the core pluripotency network. These epigenetic distinctions were accompanied by transcriptomic differences: clusters upregulated modules related to cell–cell adhesion, DNA replication, and proliferative pathways, whereas single CTCs favored metabolic stress and RNA-processing programs. Importantly, immunostaining confirmed higher levels of the proliferation marker Ki67 in clustered CTCs compared with single cells, validating

their transcriptional hyperproliferative phenotype. These findings indicate that the metastatic potency of homotypic clusters reflects not only structural cohesion but also profound epigenetic and transcriptional reprogramming.

The recognition that clusters are highly metastasis-competent raises the question of whether they can be selectively targeted. To this end, we conducted a large-scale screen of 2,486 FDA-approved compounds to identify agents capable of disrupting cluster integrity. Among these, cardiac glycosides and Na^+/K^+ ATPase inhibitors (e.g., ouabain, digoxin) were found to effectively dissociate clusters into single cells (14). Dissociation was accompanied by reversal of cluster-specific hypomethylation signatures and suppression of metastatic seeding in mouse models. This finding is conceptually significant for two reasons. First, it demonstrates that the physical state of carcinoma cells – clustered *versus* single – directly shapes their epigenetic landscape and metastatic competence. Second, it provides a therapeutic avenue: pharmacological dissociation of clusters may blunt metastasis by reverting cells to a less aggressive epigenetic state.

The convergence of experimental and clinical data positions homotypic CTC clusters as both biomarkers and therapeutic targets in carcinoma. Their detection in patient blood is consistently associated with poor outcomes, making them valuable prognostic indicators. Moreover, their rarity yet high metastatic yield underscores the increased risk they pose in cancer progression. From a conceptual standpoint, clusters challenge classical models of metastasis centered on single-cell dissemination. Instead, they highlight the persistence of epithelial features, collective survival strategies, and stemness-like reprogramming as more efficient routes to colonization. Clinically, interventions that combine liquid biopsy detection of clusters with cluster-targeting therapies may represent a new opportunity in metastasis prevention. For patients with oligometastatic disease or minimal residual disease following local therapy, intercepting cluster-mediated dissemination could delay or prevent progression to widespread, incurable metastasis.

Heterotypic CTC clusters

For the reasons provided above, CTC biology has evolved from an early focus on solitary carcinoma cells to a broader recognition that tumor cells can circulate as multicellular aggregates. While homotypic clusters – composed exclusively of carcinoma cells – have been established as highly efficient metastatic seeds in breast cancer, recent work from our team as well as others has expanded this paradigm by demonstrating that tumor cells can also circulate as heterotypic clusters, in which malignant cells travel in concert with immune or stromal partners. Among these, clusters containing neutrophils have emerged as particularly potent drivers of metastasis (15) focus is often given to interactions that occur within the primary tumor and its microenvironment, whereas the role of immune cells during cancer dissemination in patients remains largely uncharacterized 2,3. Circulating tumor cells (CTCs. Our study provided the first detailed characterization of heterotypic CTC clusters in patients and preclinical models, revealing that tumor-immune cell interactions in circulation create a cooperative niche that profoundly enhances metastatic potential. This chapter synthesizes the major findings of that work, highlighting the biology, molecular features, and clinical implications of heterotypic CTC clusters ***(Fig. 2).***

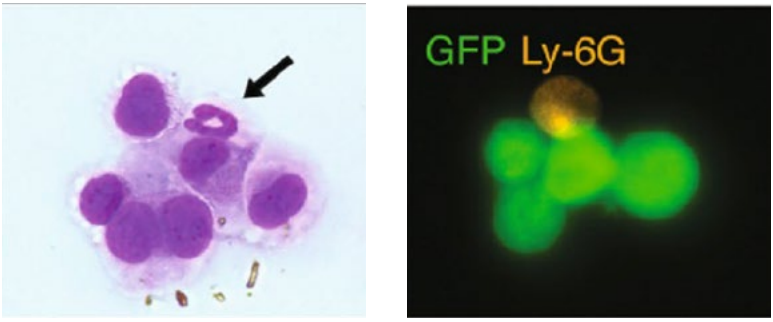

Figure 2*. Representative immunofluorescence images of CTC–neutrophil clusters isolated from a breast cancer mouse model and stained for Ly-6G* ***(gold)****. CTCs stably express GFP* ***(green) (left).*** *Representative images of CTC–neutrophil clusters stained with the Wright-Giemsa protocol to highlight nuclear morphology. The arrow points to the nucleolus of a neutrophil, appearing as multi-lobulated* ***(right)****.*

Using single-cell resolution imaging of patient blood samples, we documented the existence of CTC clusters containing both tumor cells and neutrophils in breast cancer. Although heterotypic clusters represented a minority of all CTC events, their presence was consistent across multiple individuals, confirming that they are a reproducible features of carcinoma dissemination. Importantly, the frequency of these heterotypic clusters correlated with poor clinical outcomes (15). Patients with detectable CTC-neutrophil clusters exhibited significantly shorter progression-free and overall survival compared to those with only single CTCs or homotypic clusters. These clinical correlations underscored the functional significance of heterotypic interactions and prompted mechanistic exploration of how neutrophils potentiate metastasis. Neutrophils, the most abundant leukocytes in circulation, are traditionally regarded as first responders of innate immunity. However, accumulating evidence has implicated them in tumor progression through pro-angiogenic, immunosuppressive, and metastasis-promoting roles. In the context of CTC clusters, we demonstrated that neutrophils directly enhance the metastatic fitness of tumor cells. In experimental metastasis assays, heterotypic clusters containing neutrophils displayed markedly higher lung colonization efficiency compared with homotypic clusters or single cells. Genetic and pharmacological depletion of neutrophils significantly reduced the formation of heterotypic clusters and suppressed metastatic burden in mouse models, firmly establishing neutrophils as active facilitators (rather than passive bystanders) in dissemination. Mechanistically, neutrophils conferred survival and proliferative advantages to associated tumor cells in circulation. Transcriptomic analyses uncovered that tumor cells in heterotypic clusters upregulated gene programs linked to proliferation and survival, indicating that neutrophil association reprograms carcinoma cells toward a more aggressive phenotype.

We then employed single-cell RNA sequencing to delineate the molecular features of tumor cells and neutrophils within heterotypic clusters. A distinct transcriptional signature emerged, defined by upregulation of genes involved in cell cycle progression and DNA replication in tumor cells bound to neutrophils. These programs contrasted with the stress-response signatures observed in single CTCs, suggesting that heterotypic interactions directly promote proliferative competence. Neutrophils

within clusters also displayed unique transcriptional state. Cluster-associated neutrophils upregulated survival-supporting genes, including cytokines and chemokines such as IL6 and IL1b, capable of sustaining tumor cell growth (15). This bidirectional communication established a cooperative circuit in which both tumor and immune partners adopted pro-metastatic phenotypes. Further, depletion of neutrophils using anti-Ly6G antibodies drastically reduced the abundance of heterotypic clusters and resulted in lower metastatic colonization in the lungs. This experiment demonstrated causality: heterotypic clusters are not merely markers of aggressive disease but active mediators of metastasis.

Interestingly, tumor cells within heterotypic clusters demonstrated enhanced proliferative and stemness markers, reminiscent of the stemness-associated methylation signatures previously described in homotypic clusters. This convergence suggests that collective dissemination, whether homotypic or heterotypic, systematically endows carcinoma cells with properties that favor colonization and outgrowth.

Hypoxia and CTC clusters generation

An outstanding question in the field has been how CTC clusters are generated. Hypoxia, defined as reduced oxygen availability in tissues, is a pervasive feature of solid tumors and a critical driver of malignant progression. Due to rapid cellular proliferation and aberrant vascularization, carcinomas frequently develop regions of low oxygen tension, which activate adaptive transcriptional programs that promote angiogenesis, metabolic plasticity, invasion, and resistance to therapy. Within this hypoxic microenvironment, carcinoma cells undergo profound phenotypic changes that not only enhance survival but also facilitate dissemination. Recent work from our lab has highlighted an important role of hypoxia in the generation of CTC clusters, both homotypic and heterotypic, providing new mechanistic insight into how microenvironmental cues within the primary tumor influence metastatic dissemination ***(Fig. 3)*** (16).

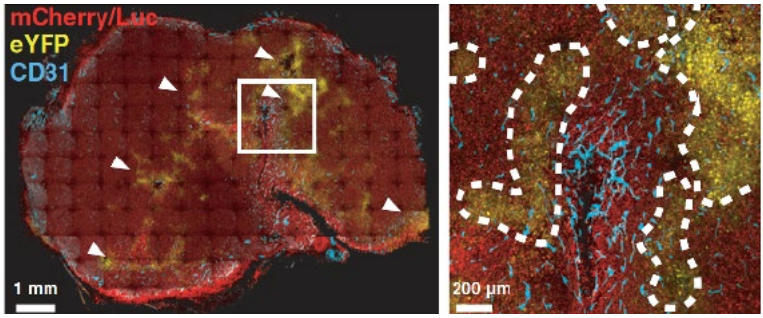

***Figure 3**. Representative immunofluorescence staining of a breast tumor section, highlighting hypoxic regions. White arrows point to hypoxic tumor regions (**yellow** fluorescent protein, eYFP), alongside staining for cancer cells (**red,** expressing mCherry-Luciferase) and blood vessels (**blue,** CD31) (**left**). The white square (**left**) demarcates a region of the tumor that is shown at higher magnification, and where hypoxic regions are circled with a white dashed line (**right**). Blood vessels are present in both hypoxic and normoxic tumor regions, with higher representation in normoxic areas (**right**).*

Using both patient-derived samples and experimental tumor models, we demonstrated that hypoxia directly increases the frequency of CTC clusters (16). Hypoxic regions within tumors were found to be preferential sites of cluster shedding, suggesting that microenvironmental oxygen deprivation acts as a trigger for collective dissemination. Mechanistically, hypoxia promoted cell–cell adhesion and cooperative migration, enabling groups of carcinoma cells to detach and intravasate as cohesive units. This effect was mediated in part through the transcriptional activation of adhesion molecules and junctional proteins, under the control of hypoxia-inducible factor (HIF) signaling. Thus, hypoxia does not simply enhance tumor invasiveness in a general sense; it programs tumors to shed multicellular aggregates, directly seeding the systemic circulation with clusters that are highly competent for metastasis. The biological significance of hypoxia-induced clusters was evaluated through functional assays. We demonstrated that clusters generated under hypoxic conditions exhibited markedly higher metastatic seeding efficiency than those originating from normoxic regions (16). *In vivo* models showed that hypoxia-driven clusters colonized distant organs more effectively, leading to increased metastatic burden. Intrinsically, hypoxia-induced clusters dis-

played upregulation of survival pathways, stemness-associated genes, and cell-cycle regulators, mirroring the transcriptional profiles observed in previous studies of CTC clusters.

The recognition that hypoxia drives CTC cluster formation has important clinical ramifications. First, it provides a mechanistic explanation for the well-established correlation between tumor hypoxia and poor patient prognosis. Hypoxia not only promotes local aggressiveness but also fuels systemic dissemination *via* clusters, which are largely effective at generating metastases. Second, it raises the possibility that hypoxia-targeted therapies may reduce metastatic risk by suppressing cluster formation. Pharmacological agents that destabilize HIF signaling or normalize tumor vasculature could potentially decrease the frequency of cluster shedding, thereby limiting systemic dissemination. Third, the findings highlight the potential value of integrating hypoxia biomarkers with liquid biopsy approaches. Monitoring hypoxia-induced transcriptional or epigenetic signatures in CTCs could enable clinicians to identify patients at heightened risk of cluster-mediated metastasis, guiding therapeutic decision-making. More broadly, these findings provide a unifying mechanistic link between the hypoxic tumor microenvironment and the systemic spread of carcinoma.

Timing of CTC formation

The dissemination of carcinoma cells through the bloodstream is the defining step of the metastatic cascade, enabling malignant cells to seed distant organs. Traditionally, the release of CTCs has been considered a continuous process, driven by intrinsic tumor biology and microenvironmental cues such as hypoxia or interactions with stromal and immune partners. However, recent evidence has challenged this paradigm, revealing that CTC release is not constant but subject to temporal regulation. In a recent study, we demonstrated that the timing of CTC formation in breast cancer is intricately linked to circadian rhythms, uncovering a previously unrecognized dimension of cancer cell dissemination (17).

Circadian rhythms are endogenous, approximately 24-hour cycles that regulate a wide range of physiological processes, including metabolism, hormone secretion, immune function, and cell cycle progression. They are or-

chestrated by transcriptional-translational feedback loops of core clock genes, such as CLOCK, BMAL1, PER, and CRY, which maintain systemic and tissue-specific oscillations. In cancer biology, circadian disruption has been implicated in tumor initiation and progression. Epidemiological studies have associated shift work and chronic circadian misalignment with increased cancer risk, while experimental data show that clock gene dysregulation alters tumor growth and therapeutic responses. Despite these links, the role of circadian rhythms in metastasis has been unclear.

We conducted time-resolved blood sampling in breast cancer patients and corresponding xenograft models to determine whether CTC release followed temporal patterns. Strikingly, we found that the majority of CTCs were released during the rest phase of the circadian cycle. In humans, this corresponded to the nighttime period, while in nocturnal mouse models, it aligned with daytime rest. This finding challenged the long-standing assumption of constant CTC release. Instead, dissemination was shown to be temporally gated, with peaks during specific circadian phases (ca. 4 h upon sleep initiation). Quantitatively, up to 80% of detectable CTCs were found during the rest phase, suggesting that circadian timing is a dominant regulator of systemic tumor cell shedding ***(Fig. 4).***

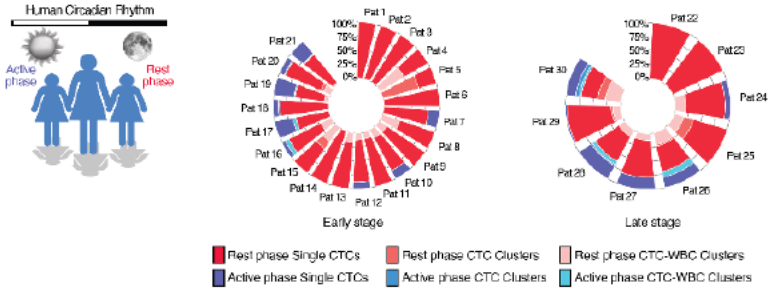

Figure 4*. Most CTCs are found during sleep in breast cancer patients. Graphical representation of the human circadian rhythm. The white and black bars represent environmental light (active period) and dark (rest period) conditions, respectively* ***(left).*** *Radial histograms showing the percentage of single CTCs, CTC clusters and CTC–WBC clusters isolated during the rest and active phases in patients with early- or late-stage breast cancer* ***(left).***

Mechanistically, we found that fluctuations in hormonal signaling, particularly glucocorticoids, insulin, and androgens, contributed to the rhythmicity of CTC dynamics. These systemic cues modulated tumor cell proliferation, adhesion, and intravasation, creating time windows of heightened dissemination. At the cellular level, tumor cells collected during peak release phases showed transcriptional upregulation of genes involved in cell cycle progression, DNA replication, and mitotic activity, indicating that proliferative states were temporally synchronized with systemic circadian signals. In essence, breast carcinoma cells were not only more likely to intravasate during the rest phase but were also intrinsically more proliferative and invasive at those times. Of note, blocking hormonal cues disrupted the circadian oscillation of CTC release, demonstrating that systemic endocrine rhythms directly influence tumor dissemination. Beyond enumeration, we assessed the functional competence of CTCs collected at different times of day. Remarkably, CTCs harvested during the rest phase exhibited greater metastatic seeding potential in experimental models compared with those collected during the active phase. This finding emphasized that circadian timing influences not only the quantity but also the metastatic competence of CTCs. Thus, rest-phase CTCs in breast cancer were enriched for proliferative, stemness-associated transcriptional signatures that conferred heightened metastatic efficiency. These insights extend the emerging picture from prior studies: just as cluster formation or hypoxia-induced shedding enhances metastatic potential, circadian timing determines when the most aggressive cells are released into circulation.

The circadian regulation of CTC dynamics carries several important clinical implications. First, diagnostic timing matters. Blood draws used for liquid biopsy, CTC enumeration, or molecular profiling may yield vastly different results depending on the time of day. Sampling during active versus rest phases could introduce biases, potentially skewing prognostic assessments. Standardizing sampling times, or at least accounting for circadian variation, may therefore improve the reliability of CTC-based diagnostics. Second, treatment timing may be optimized. The field of chronotherapy, which tailors drug administration to circadian cycles, has shown promise in improving efficacy and tolerability of chemotherapy as well as other agents. Our findings suggest that synchronizing therapy

with CTC release dynamics could enhance anti-metastatic effects. For example, administering drugs during periods of peak dissemination might more effectively intercept circulating cells. Third, endocrine manipulation may reduce dissemination. Since hormonal cues mediate circadian CTC rhythms, pharmacological modulation of these pathways could dampen dissemination. Agents that blunt circadian hormonal oscillations may thereby reduce the systemic release of metastasis-competent cells.

The recognition of circadian timing adds a temporal dimension to the spatial and molecular heterogeneity already described in CTC biology. Homotypic clusters demonstrate how cell–cell adhesion and epigenetic reprogramming boost metastatic potential; heterotypic clusters illustrate the extrinsic support provided by immune partners; hypoxia reveals how microenvironmental cues drive cluster formation; and now these insights depict metastasis as a spatiotemporally dynamic process shaped by circadian rhythms. Understanding this complexity is essential for designing therapeutic strategies that can effectively target dissemination.

Targeting CTC clusters

Emerging insights into CTC cluster biology have revealed them as critical drivers of metastasis – capable of resisting blood circulation dynamics while conveying stemness and proliferative traits. Recognizing these clusters as a major Achilles' heel opens the door for therapeutic disruption. In a recent proof-of-concept clinical trial, we directly targeted this vulnerability by administering the Na^+/K^+ ATPase inhibitor digoxin to patients with metastatic breast cancer, providing the first in-human demonstration that CTC clusters can be pharmacologically dismantled (18).

In a collaboration between ETH Zurich, University Hospitals of Basel and Zurich, and Basel-Land Cantonal Hospital, we initiated a prospective, open-label Phase I study aiming to assess whether low-dose digoxin could reduce the size of CTC clusters in patients with metastatic breast cancer. Nine women received daily digoxin treatment over one week, while a matched, non-randomized control group was untreated. The study successfully met its primary endpoint: a statistically significant average

reduction of 2.2 cells per cluster, from a baseline cluster size of approximately four cells. Both homotypic and heterotypic clusters were affected ***(Fig. 5)*** (18).

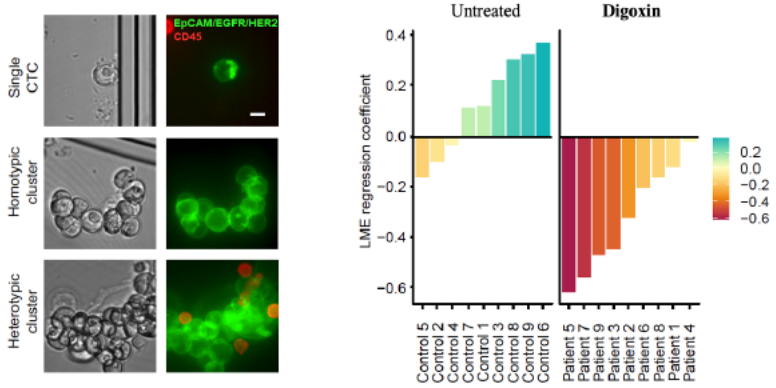

Figure 5*. Treatment with digoxin reduces CTC cluster size in patients with breast cancer. Representative images of a single CTC and homotypic and heterotypic CTC clusters (scale bar, 10 μm), stained with EpCAM, HER2 and EGFR (green) and CD45 (magenta)* ***(left).*** *LME random coefficients showing a negative association between treatment and the average size of CTC clusters, including both homotypic and heterotypic units* ***(right).***

Importantly, no treatment-related adverse events occurred, indicating a favorable safety profile at the administered dose. Digoxin inhibits the Na^+/K^+ ATPase, a membrane ion pump critical for maintaining cellular electrochemical balance. Its inhibition leads to increased intracellular calcium levels, which disrupts cell–cell adhesion machinery in clusters. Transcriptomic profiling of CTCs post-treatment revealed downregulation of adhesion-related and cell cycle–associated genes, aligning with observed cluster dissolution effects in mouse models. This study represents the first demonstration in humans that CTC cluster integrity is a druggable vulnerability.

Although still a proof-of-mechanism in patients, this study marks an important step forward, as several avenues now emerge that can shape the

future of CTC cluster-targeted therapy. One clear direction is the refinement of small molecules that act on the mechanisms of cluster cohesion, ideally with greater specificity for tumor-derived cell–cell adhesion pathways and a reduced risk of systemic toxicity compared with classical Na^+/K^+ ATPase inhibitors. In parallel, the systematic use of serial blood sampling and high-resolution liquid biopsy technologies will be essential to monitor how cluster dynamics evolve in response to treatment, and to determine whether early reductions in cluster size or frequency can serve as reliable surrogate markers of therapeutic efficacy. Another critical step will be the design of multimodal clinical trials in which cluster-targeting agents are administered alongside established systemic therapies, including cytotoxic drugs, targeted inhibitors, or immunotherapies. Such combined regimens may be particularly valuable in oligometastatic disease, where the prevention of new metastatic lesions could substantially prolong overall survival. Moreover, integration with emerging biomarkers – for instance, cluster-derived transcriptional or epigenetic signatures – may allow patient stratification and enable personalized approaches.

Equally promising is the prospect of aligning cluster-targeting interventions with the temporal and microenvironmental drivers of dissemination described in earlier studies. For example, dosing schedules could be synchronized with circadian peaks of CTC release to maximize the likelihood of intercepting clusters at their most vulnerable stage. Similarly, the combination of cluster-disrupting agents with therapies that target hypoxia-driven dissemination or neutrophil-mediated heterotypic clustering may provide synergistic effects by undermining multiple converging pathways of metastatic competence.

Taken together, these strategies chart a forward-looking agenda in which cluster biology is no longer viewed solely as a marker of disease aggressiveness but as a therapeutic target. The translation of this concept into larger, more definitive clinical studies will determine whether the dissolution of CTC clusters can indeed be leveraged into a durable anti-metastatic strategy capable of altering the natural course of carcinoma.

Summary and conclusions

Metastatic disease remains the principal cause of mortality in carcinomas, and its biology continues to reveal layers of complexity that both challenge and inspire therapeutic opportunities. Across the preceding chapters, we have traced an evolving understanding of how carcinoma cells disengage from primary tumors, traverse systemic circulation, and colonize distant organs. The journey from primary lesion to overt metastasis is neither linear nor uniform; rather, it is shaped by the interplay of tumor-intrinsic traits, microenvironmental stressors, systemic host physiology, and, as the most recent clinical data seem to suggest, vulnerabilities that can be therapeutically exploited.

The earliest insights into CTC biology positioned solitary carcinoma cells as the canonical metastatic intermediates. However, the recognition that tumor cells may travel collectively – whether as homotypic aggregates of carcinoma cells or heterotypic consortia with immune partners – marked a shift in perspective. Homotypic clusters were shown to possess unique transcriptional and epigenetic landscapes that confer stemness and proliferative fitness, enabling them to seed metastases with higher efficiency compared with single CTCs. Heterotypic clusters, particularly those incorporating neutrophils, further expand this picture by demonstrating how tumor–immune cooperation in circulation actively potentiates metastatic colonization in breast cancer. Together, these findings underscore that metastasis is rarely the endeavor of isolated cells; rather, it is a collective process fueled by both intrinsic and extrinsic cooperation. Equally important has been the recognition of upstream drivers of cluster formation. Hypoxia, a pervasive feature of disorganized tumor vasculature, not only promotes local invasion but also instructs tumors to disseminate as clusters. By stabilizing HIF transcription factors, hypoxia reinforces cell–cell adhesion while activating survival and stemness programs, thereby producing highly competent metastatic seeds. Temporal dynamics add yet another dimension: the discovery that CTC release is gated by circadian rhythms challenges the notion of constant dissemination and introduces time as a critical regulator of metastatic potential. Carcinoma cells are not shed randomly but preferentially during the host's rest phase,

when endocrine and proliferative cues converge to produce CTCs of heightened aggressiveness.

Taken together, these mechanistic insights converge on the realization that CTC clusters – whether homotypic, heterotypic, hypoxia-driven, or temporally synchronized – are central agents of metastatic progression in breast cancer, and possibly in other cancer types. This recognition naturally raises the question of whether they can be therapeutically targeted. Our recent proof-of-concept clinical trial provides first evidence in this regard. By administering the Na^+/K^+ ATPase inhibitor digoxin, we demonstrated that pharmacological disruption of cluster integrity is not only feasible in patients but can be achieved safely, leading to measurable reductions in cluster size. Although preliminary and limited in scope, this study shifts the conversation from descriptive biology to actionable intervention, opening a new opportunity in metastasis-directed therapy.

Looking forward, the integration of these discoveries charts a compelling agenda. The biology of CTC clusters must be interrogated not only in terms of structure and composition, but also in their dynamic regulation by microenvironmental cues and circadian timing. Future clinical strategies will need to incorporate this complexity, perhaps by combining cluster-targeting agents with therapies that modulate hypoxia or immune-mediated interactions, and by aligning drug administration with circadian windows of peak dissemination. If successful, such approaches could convert the current descriptive understanding of cluster biology into tangible benefits – for instance delaying or preventing metastasis, prolonging survival, and ultimately altering the natural history of carcinoma.

In sum, the study of CTC clusters has transitioned from a biological curiosity to a translational opportunity. They embody the convergence of tumor autonomy and host physiology, and they now represent a new opportunity for drug development. Future clinical studies will be key to determine whether CTC clusters targeting results in survival benefits for patients with carcinomas.

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1986	Prof. Dr. Walter Schaffner Prof. Dr. Ueli Schibler
1987	Prof. Dr. Jacques Louis Prof. Dr. Joachim H. Seelig
1988	Prof. Dr. Jean-Dominique Vassalli PD Dr. Hans Hengartner
1989	Prof. Dr. Heini Murer Dr. Hugh Robson MacDonald

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1992	PD Dr. Leena Kaarina Bruckner-Tuderman Prof. Dr. Jürg Tschopp	
1993	Dr. Paolo Meda Prof. Dr. Adriano Fontana Prof. Dr. Michel Aguet	} Jubiläumsjahr
1994	Prof. Dr. Hans Rudolf Brenner Prof. Dr. Daniel Pablo Lew	
1995	Prof. Dr. Jürg Reichen Dr. George Thomas jr.	
1996	Dr. Lukas C. Kühn Prof. Dr. Peter Sonderegger	
1997	Dr. Gérard Waeber Prof. Dr. Denis Duboule	
1998	Prof. Dr. Adriano Aguzzi Prof. Dr. Primus E. Mullis	
1999	Prof. Dr. Clemens A. Dahinden Prof. Dr. Antonio Lanzavecchia	
2000	Prof. Dr. Giuseppe Pantaleo Dr. Brian A. Hemmings	
2001	Prof. Dr. Isabel Roditi Dr. Thierry Calandra	
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2005	Prof. Dr. Urs Emanuel Albrecht Prof. Dr. Dominique Muller
2006	Prof. Dr. Adrian Merlo Prof. Dr. Michael O. Hengartner
2007	Prof. Dr. François Mach Prof. Dr. Nouria Hernandez
2008	Prof. Dr. Darius Moradpour Prof. Dr. Sabine Werner
2009	Prof. Dr. Margot Thome-Miazza Prof. Dr. Walter Reith
2010	Prof. Dr. Christian Lüscher Prof. Dr. Burkhard Becher
2011	Prof. Dr. Petra S. Hüppi
2012	Prof. Dr. Olaf Blanke
2013	Prof. Dr. Andreas Papassotiropoulos Prof. Dr. Dominique J.-F. de Quervain
2014	Prof. Dr. Marc Y. Donath Prof. Dr. Henrik Kaessmann
2015	Prof. Dr. Dominique Soldati-Favre Prof. Dr. Fritjof Helmchen
2016	Prof. Dr. Michel Gilliet Prof. Dr. Andreas Lüthi

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2019	Prof. Dr. Botond Roska Prof. Dr. Oliver Distler
2020	Prof. Dr. Mohamed Bentires-Alj Prof. Dr. Nadia Mercader Huber
2021	Prof. Dr. Bart Deplancke Prof. Dr. Anne Müller
2022	Prof. Dr. Doron Merkler Prof. Dr. Annette Oxenius
2023	Prof. Dr. Christoph Hess Prof. Dr. Sebastian Jessberger
2024	Prof. Dr. Andrea Alimonti Prof. Dr. Andrea Ablasser
2025	Dr. Virginie Hamel und Prof. Dr. Paul Guichard Prof. Dr. Nicola Aceto