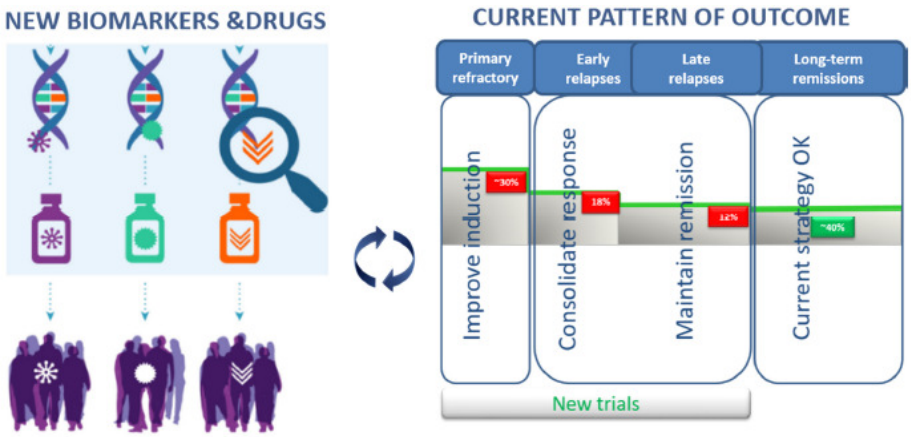
*i*Lymph

A clinical and translational research program aiming at combining biomarker-driven and risk-adapted management approaches in patients with malignant lymphoproliferative diseases

Francesco d'Amore, MD, DrMedSc, Dept. og Hematology, Aarhus University Hospital

*The **right** drug for the **right** person at the **right** dose…*
The Precision Medicine Initiative Cohort Program
www.nih.gov/AllofUs-Research-Program

*…as part of the **right** management strategy for the **right** patients at the **right** time and **adapted** to tumor- and host-related **risk** profiles*
http:/sites.tufts.edu/insight/2016/12/09/precision-medicine-too-big-to-fail/



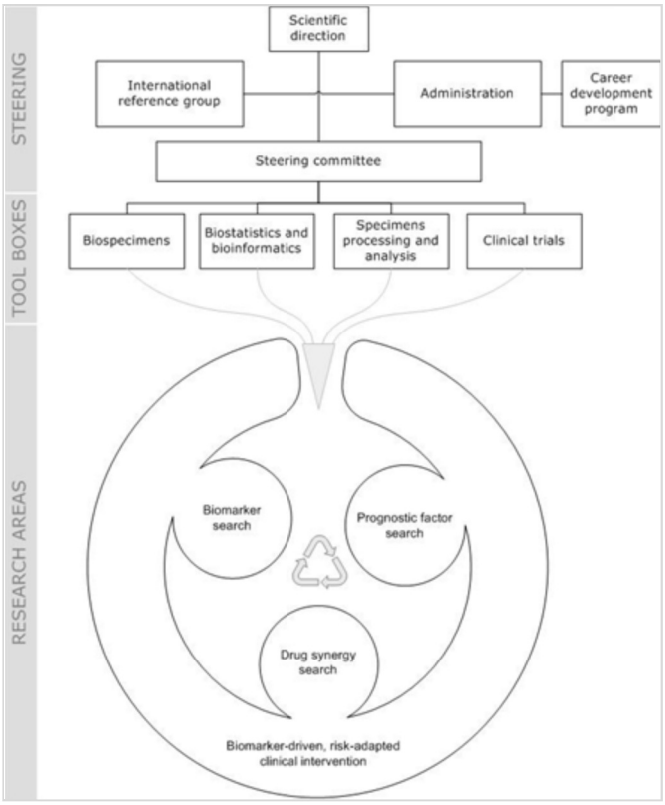
Lymphoid malignancies have become one of the most common cancers in both men and women and, if current incidence trends continue, they will soon reach the top five level. Hence, lymphoma represents a significant cause of morbidity and mortality worldwide, with an estimated 450,000 new cases and 225,000 deaths annually. It is also important to realize that lymphoma represents a heterogeneous group of diseases for which outcomes can vary greatly. For example, the indolent B-cell lymphomas, such as follicular lymphoma, marginal zone lymphoma, and small lymphocytic lymphoma, are still incurable chronic conditions, requiring patients to undergo repeated exposures to toxic therapies. On the other hand, for the more aggressive lymphomas, modern treatment regimens result in long-term survival rates ranging from over 80% for Hodgkin lymphoma

(HL) to approximately 60% for diffuse large B-cell lymphoma (DLBCL) and 40% for peripheral T-cell lymphoma (PTCL). More-effective therapeutic strategies might prevent many patients from dying of their disease. However, even for those patients who are cured, combination chemotherapy, often used in conjunction with radiation, can result in long-term toxicities that impair health, predispose patients to secondary malignancies, and negatively impact quality of life.

A better understanding of the molecular pathogenesis of lymphoma, of the host's immunological response and of the tumor biology predictive of treatment sensitivity or refractoriness, will enable the development of novel therapies as well as novel management strategies with better efficacy and less toxicity. Early detection of lymphoid malignancies along with the ability to better predict development of an aggressive versus an indolent clinical behavior are useful pre-requisites for a successful management strategy. The use of reliable biomarkers for early stage detection and for the identification/stratification of groups at risk for poorer outcome, may improve overall survival rates by reducing the high levels of morbidity and mortality associated with late stage diagnosis. Similarly, recognition of favorable biofeatures associated with very good outcomes vis-à-vis standard approaches may encourage a de-escalation of treatment intensity in some patients.

Over the past three decades, research in the lymphoma field has focused on an ever-more precise histological classification that ultimately resulted in the identification of over 60 subtypes of lymphoma. However, this approach has had minimal impact on advancing therapy, because the general treatment paradigm has remained the empiric administration of combination chemotherapy. For example, to date, the most widely used regimen for the treatment of one of the most frequent lymphoma entities, DLBCL, is R-CHOP (combination of rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone). The CHOP regimen was introduced in the early 1970s, and in the following 40 years, the only major therapeutic advancement in DLBCL has been the incorporation, within the CHOP regimen, of the CD20-directed monoclonal antibody, rituximab leading to the R-CHOP combination. Several randomized trials demonstrated that the addition of rituximab to chemotherapy improves the cure rate and overall survival of patients with DLBCL from approximately 40–45% in the pre-rituximab era to 55–60%. Despite this progress, nearly 50% of patients will have disease progression and/or relapse after R-CHOP therapy and approximately 30-35% will ultimately die of their disease. Of note, CD20 expression is required for rituximab clinical activity, but is not sufficient to predict response to treatment. Hence, the identification of additional biomarkers is needed to predict response to rituximab.

The rapidly evolving development of novel biological compounds able to target specific molecular pathways with increasing precision and decreasing collateral damages makes the need for reliable biomarkers predictive of treatment response urgent. At the same time, one must be aware of the limitations of precision medicine primarily due to tumor heterogeneity and target variability among subclones. Clinical trials of new therapeutics may often be underpowered due to unrecognized heterogeneity in disease pathogenesis among enrolled patients so that drugs that are highly beneficial for a definable subset risk to be rejected because the majority of patients in the trial fail to respond. The discovery of specific host- and/or tumor-related biofeatures can be used to identify drug targets and selectively direct those drugs towards those patients that are most likely to benefit of them. Hence, knowing the genetics of the tumor and the host and while establishing biomarkers for clinical behavior and treatment response will allow us to rationally select patient groups most likely to respond to particular treatment approaches and ultimately improve their survival and quality of life.



The *i*Lymph research program intends to develop and validate biomarkers for: (1) early detection, disease progression, and recurrence of lymphoid malignancies; and, (2) risk assessment of patients with lymphoid malignancies (of B- or T-cell origin) in both the immunocompetent and immunodeficient patient. This also encourages the development and improvement of specific technologies and methods for quantitative detection of novel biomarkers associated with these diseases. The principles contained in the *i*Lymph initiative are in accordance with the NCI's Strategic Plan to support studies to better understand risk reduction, prevention, early detection, diagnosis, and treatment. The *i*Lymph projects will encompass proteomic, genomic, epigenomic, and transcriptomic analyses, as well as the utilization of standardized biospecimens for validation studies.

Organization

The organization of the *i*Lymph project relies on three main clusters of activities as shown in Fig.1 (i) interdisciplinary projects, (ii) methodological toolboxes and (iii) coordinating and administrative functions.
The structure of the *i*Lymph strategic research project will rely on four basic domains: search for predictive biomarkers, search for prognostic factors, personalized, risk-adapted clinical intervention. The translational results will be evaluated for clinical implementation in phase I, II or III trials, some of which are ongoing. The clinical trial activity will be coordinated and supported by the academic clinical trial office (A-CTO) linked to *iLymph* and located at the Dept. of Hematology, AUH.

Biospecimens: The specific aims of the biospecimens toolbox are to (i) collect, process, distribute, and bank cells from fresh tumor, frozen tumor tissue, paraffin-embedded tumor tissue, serum/plasma, and genomic DNA from patients with malignant lymphomas, and (ii) track all specimens in the *iLymph* lymphoma biobank. All material is collected and processed under tight quality control, and banked for ongoing and future research projects in accordance with ethic regulatives. The biobank is located at the Haemodiagnostic Laboratory of the Dept. of Hematology, AUH.

Specimens' processing and analysis: The specimens' processing and analysis toolbox serves as a resource of expertise collaborative support, and service for projects involving processing and analysis for, e.g: (i) pathology review/classification, (ii) immuno-histochemistry, (iii) (fluorescent) in situ hybridization, (iv) tissue arrays, (v)

digital image analysis, (vi) laser capture microdissection, (vii) next generation sequencing and other nucleic acid analyses, (viii) proteomics and other protein analyses. The Specimens' Processing and Analysis toolbox involves laboratories at the Dept. of Pathology, AUH (tissue array, immunohistochemistry, digital imaging analysis); Dept. of Endocrinology, AUH (immunoaasay for soluble PD1, sPDL1, sPDL2); Institute of Biomedicine, AU (mass spectrometry and Western blot); Dept. of Molecular Pathology, City of Hope, Duarte, CA, USA (Next generation sequencing and other nucleic acid analyses); Dept. of Molecular Pathology, Mayo Clinic, Rochester Minnesota (DUSP22 and TP63 rearrangement analysis) and Dept. of Molecular Pathology, Cornell University, New York, NY (whole exome sequencing and targeted sequencing).

Statistics, Biostatistics and Bioinformatics: The Statistics, Biostatistics and Bioinformatics toolbox provides statistical and data management support for each of the scientific *i*Lymph projects and the Administrative toolbox. Moreover, it is responsible for establishing the infrastructure to enable the link between the clinical and research databases of the different *i*Lymph projects. The comprehensive nature of this facility assures each *i*Lymph investigator access to statistical and bioinformatics expertise that includes collaborative development of study designs and analysis plans, data analysis and interpretation, abstract and manuscript preparation. This toolbox complements and assists the efforts of the Biospecimens and Clinical Trials toolboxes by providing experience with nation-wide, population-based clinical data and tissue registries.

Clinical Trials: The primary objective of the Clinical Trials toolbox is to provide assistance to the projects in all aspects of clinical trial design, implementation, regulation, elaboration of electronic clinical report forms (eCRFs), data collection, data monitoring and interpretation, pharmacokinetics, and other specimen collections for targeted therapy investigations and/or banking. Specifically, the Clinical Trials toolbox facilitates protocol writing and preparation, submission, and regulatory review. Moreover, it maintains, in close collaboration with the Biostatistics and Bioinformatics toolbox, the database and all aspects of data management and monitoring for patients enrolled on *i*Lymph endorsed clinical trials. Adverse event reporting and data together with safety monitoring for all protocols are coordinated through the Clinical Trials toolbox. It also interacts with the Biospecimens toolbox, providing clinical, epidemiologic and demographic data on patients consenting to tissue banking. Since 2016 anew 'Academic Clinical Trial Office' (A-CTO) has been established to assist the growing number of investigator-initiated trials that iLymph is involved in close partnership with the Nordic Lymphoma Group. The A-CTO is led by Head clinical trial manager, PhD Helle Toldbod and prof. Francesco d'Amore. It also comprises clinical trial manger, RN Rikke Lundkvist and secretary Malene Møller Staal.

Administration: The major function of the Administrative toolbox is to provide an organizational structure through which the investigators can interact and communicate with each other to foster the fundamental goal of the *i*Lymph project, i.e., translation of basic biological information to and from the clinical interventional platform. The Administrative toolbox schedules and coordinates all internal and external meetings, creates and circulates minutes for those meetings, and coordinates the travel for investigators and consultants. Other mechanisms of communication within the *i*Lymph network, including regular conference calls, meetings, videoconferences etc., will also be organized through the Administrative facility.

The *iLymph* research team

The scientific team of researchers and admintrative personnel presently attached to the iLymph project is shown in the table below.

Titel/Navn/Tilknytning	Starttidspkt/status	Emne

Professorat		
Dr.med. Francesco d'Amore	Overlæge, professor, leder af A-CTO, (Dr.med. 2000)	Lymfomprofessorat; leder af iLymph
Postdoktorale seniorforskere		
Cand.scient. PhD Helle Toldbod	Leder af A-CTO (PhD 2003)	Nordisk CTO til akademiske trials, GCP uv.
Cand.med. PhD Judith Jørgensen	Overlæge (PhD 2005)	Klinisk forskning i DLBCL
Cand.med. PhD Peter Kamper, Hæm.	Overlæge (PhD 2011)	Mikromiljø ved HL
Cand.scient. PhD Maja Ludvigsen	Lektor (PhD 2014)	Proteomics ved lymfomer
Cand.med. PhD Martin B. Pedersen	Post doc, HU læge (PhD 2014)	PTCL : klassificering og prognosticering
Cand.med. PhD Maja Vase	Post doc, HU læge (Phd 2015)	Mikromiljø ved ID associerede lymfomer
PhD i gang		
Cand.med. Charlotte Madsen, Hæm.	Phd projekt (i gang fra 1.3.15)	Transformerede follikulære lymfomer
Cand.med. Michael Clausen, Hæm.	Phd projekt (i gang fra 1.5.15)	Prognostiske faktorer ved DLBCL
Cand.med. Johanne Holst, Hæm.	Phd projekt (i gang fra 1.2.16)	PTCL med myelo-lymfoide egenskaber
Eksternt knyttet		
Cand.med. Lena Modvig	Forskningsprojekt	Late relapses in DLBCL and HL
Forskningsår/bachelor		
Stud.med. Julie B. Mortensen	Forskningsår (1.2.17-31.1.18)	s-PD1, PDL1, PDL2 ved hæm.sygdomme
Mol.med. Ida M.Hansen	Bachelor opgave (juni 2017)	Soluble PD1 (PDL1, PDL2) – Assay etablering
Stud.med. forksningsprojekter		
Stud.med. Franziska Szelat	Stud.med. 8.-9. semester	Upfront HDT ved PTCL
Stud.med. Emma Forsberg	Stud.med. 8.-9. semester	Primært testislymfom (DLBCL)
A-CTO personale		
Trial manager, RN Rikke Lundkvist	Clinical Trial Manager	Diverse trials, CRF i Open Clinica
Sekretær Malene Møller Staal	Sekretariatsfunktion	iLymph professorat, projektkonti, A-CTO

In addition, the *i*Lymph project has a lead coordinator (Francesco d'Amore, dept. of Hematology, AUH), who holds a clinical professorship in malignant lymphoproliferative diseases initiated by an original 5-year grant from The Karen Elise Foundation (2010-2015) and later supported by the Central Region of Denmark Research Fund and Aarhus University (AUH/AU). This professorship is the platform upon which the *i*Lymph project is built. Moreover, the research network counts several senior scientists from different disciplines, who interact

on one or several topics as co-supervisors according to the specific needs and features of the individual research projects. These senior scientists are research partners as well as co-supervisors from various fields such as pathology (profs. Stephen Hamilton-Dutoit and Torben Steiniche,Trine Plesner AUH, dr. Peter Nørgaard, Herlev Hospital, Michael B. Møller, OUH), medical biochemistry (prof.Bent Honorè, AU), molecular biology and molecular genetics (prof. Reiner Siebert, Dept. of Genetics, Kiel University), infectious medicine (dr. Carsten Schade, AUH), nephrology (prof. Bente Jespersen, AUH), epidemiology (prof. Henrik Toft Sørensen, dr. Mette Nørregaard, KEA, AUH, dr. Henrik Hjalgrim, SSI) bioinformatics (dr. Søren Besenbacher, BIRC, AU) in- and outside AU/AUH.

The projects

All scientific activities taking place within the *i*Lymph project develop and interact within the framework of a 'project matrix' consisting of two major domains, one represented by disease-specific projects and the other by cross-sectional topics applicable within the different disease-specific fields. Each field or topic is related to an individual scientist or research team and may contain one or more individual projects, which, according to understandable dynamics, will be either ongoing or in pipeline. There are presently 5 disease-defined fields within iLymph. They are : (1) Hodgkin lymphoma (HL), (2) Peripheral T-cell lymphoma (PTCL), (3) Diffuse large B-cell lymphoma (DLBCL), (4) Follicular lymphoma (FL) with focus on transformed follicular lymphoma(tFL) and (5) immunodeficiency-associated lymphoma (IDL) with focus on post transplant lymphoproliferative disease (PTLD) and HIV-associated lymphoma (HIV-L). Along with these 5 disease-specific fields, there are 8 cross-sectional research topics: (i) epidemiology, (ii) genomics, (iii) proteomics, (iv) microenvironment, primarily focusing on tumoral microenvironment, (v) angiogenesis, (vi) diagnostic imaging, primarily focusing on PET/CT-related issues within different types of lymphomas

Two of the ongoing projects, which illustrate the activities of the iLymph research group, are briefly described below:

The ACT-1 trial in T-cell lymphomas

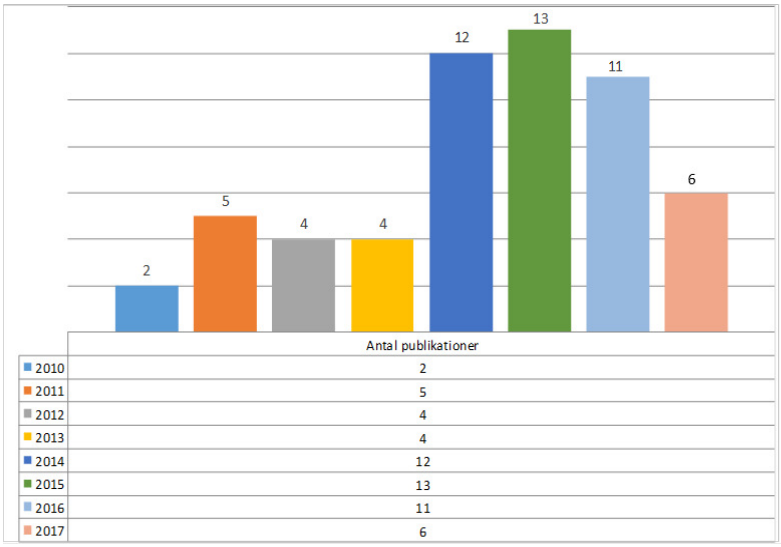
As coordinating center (Dept. of Hematology, AUH) and with Principal Investigator role, we coordinated the first international randomized trial ever conducted in transplant eligible patients (<65 yrs) with PTCL (the ACT-1 trial). The trial tested the addition of the anti-CD52 antibody alemtuzumab to a conventional chemotherapy backbone (CHOP) followed by autologous stem cell transplantation. We have collected all the available trial-related tissue specimens and plan to perform an RNA sequencing analysis in collaboration with our research partners at British Columbia Cancer Agency (dr. David Scott), Vancouver, Canada and City of Hope University (dr. Wing Chung Chan), City of Hope, CA, USA. The hope is to identify a CD52-related biofeature predictive for response to the antibody. Results of the correlative analysis will hopefully be presented at a major conference in 2017-2018.

The DUSP22/TP63 project in ALK-neg ALCL

The DUSP22/TP63 project is run in collaboration with dr. Andrew Feldman from the Dept. of Pathology at Mayo Clinic, Rochester, MN, USA. In a cohort of US patients with a type of T-cell lymphoma known as ALK-negative anaplastic large celle lymphoma (ALK- ALCL), a rearrangement of the DUSP22 gene was found to be associated with a particularly favorable outcome, while a rearrangement in the TP63 gene was associated with an ominous prognosis. Through iLymph and the Nordic Lymphoma Group it has been possible to access the tissue microarray samples of one of iLymphs phd projects (post-doc dr. Martin Bjerregaard Pedersen) and the trial specimen of a former Nordic transplantation trial (trial code name: NLG-T-01). The independent analysis of both cohorts has revealed the same findings as in the US cohort. Furthermore it has provided evidence that the

DUSP-22 gene rearrangement is associated to a similar favorable outcome regardless of whether an autologous stem cell transplantation was performed after induction therapy or not. On the other hand our Danish/Nordic data also provided evidence that transplantation was still a good option for those patients that did not carry the DUSP22 rearrangement.

Publications



The number of *i*Lymph-related publications has been markedly increasing over the last years. Since 2014 there has been a publication frequency of approximately one paper pr month and the impact factor profile has also increased with first and last authorships in high ranking journals such as Blood and Journal of Clinical Oncology. Since the beginning of the professorship granted by the Karen Elise Jensen Foundation in February 2010, a total of 57 publications has been produced (see figure). Since January 2017, 6 papers have been published and further 4 manuscript are currently under active revision.

PhD degrees

Two PhD projects were successfully concluded in 2014, two in 2015 and one in 2016. Three PhD projects are currently ongoing along with one research-year student. The latter project deals with establishment of an assay for measurement of soluble PD1 and its ligand PDL1. This project is expected to yield a reliable method for the measurement of longitudinal clinical samples from the iLymph biobank and for Nordic sample sets provided through a collaboration with the Universities of Uppsala and Helsinki. This will most probably imply an expansion of the present working plan into a regular PhD project.

Annual research seminar

An annual 2-days research seminar has been held at Schur Conference Center, Glud, Denmark in May since 2010. At the seminar, young researchers have presented and discussed their work in the *i*Lymph plenum. Key note speakers were invited to give expert overviews on topics relevant to the ongoing projects. All seminars have been extremely valuable for the exchange of ideas and useful discussions on the challenges and discoveries within the different projects. We aim to continue this unifying team-building tradition.

The Nordic Meeting of Tumor Microenvironment in Lymphoma

iLymph has established since 2015 an international meeting held in Aarhus on the topic Tumor Microenvironment in Lymphoma. The meeting is chaired by prof. F.d'Amore and a colleague from the British Columbia Cancer Agency in Vancouver (prof. Randy Gascoyne in 2015 and prof. Christian Steidl in 2016). Both editions of the meeting have been a success and a 3rd edition is planned for May 2018. For further details see www.nordictumormicroenvironment.org

Perspectives

The primary goal of *i*Lymph is to improve the cure rate and the quality of life of patients suffering from lymphoid cancers through innovative therapeutic strategies based on effective translation of discoveries in lymphoma biology, immunology, and molecular genetics and on risk-adapted treatment strategies. More specifically, *i*Lymph research activities are expected to consolidate an already existing national and international network for the study of lymphoid cancers. Discoveries on the biological mechanisms and determinants of clinical behavior of lymphoid malignancies originating within the *i*Lymph network will provide the platform for collaborative trials testing novel biomarker-driven, risk-adapted therapeutic approaches on a national and international scale. The network is designed to ensure patient access to new investigational drugs and to allow easy access to phase I and early phase II programs. A prominent achievement since 2015 is the establishment of the Academic Clinical Trial Office serving as a link between the translational research carried out within the iLymph group and the clinical trial activity linked to it, which is implemented as part of the Nordic Lymphoma Group trial portfolio. The planned projects are expected to strengthen and expand existing international collaborations, attract foreign researchers, and further heighten the educational level in the research and management of hematological malignancies at our university hospital/university as well as at national level.