Institution: King's College London		
Unit of Assessment: UoA5		
Title of case study: Improving cancer treatments and patient outcomes using Fluorescence Lifetime Imaging Microscopy-Förster Resonance Energy Transfer (FLIM-FRET)		
Period when the underpinning research was undertaken: 1999 - 2018		
Details of staff conducting the underpinning research from the submitting unit:		
Name(s):	**Role(s) (e.g. job title):**	**Period(s) employed by submitting HEI:**
Tony Ng Simon Ameer-Beg	Professor Professor	2004 – present 2005 – present
Period when the claimed impact occurred: 2011 - 2020		
Is this case study continued from a case study submitted in 2014? N		

1. Summary of the impact

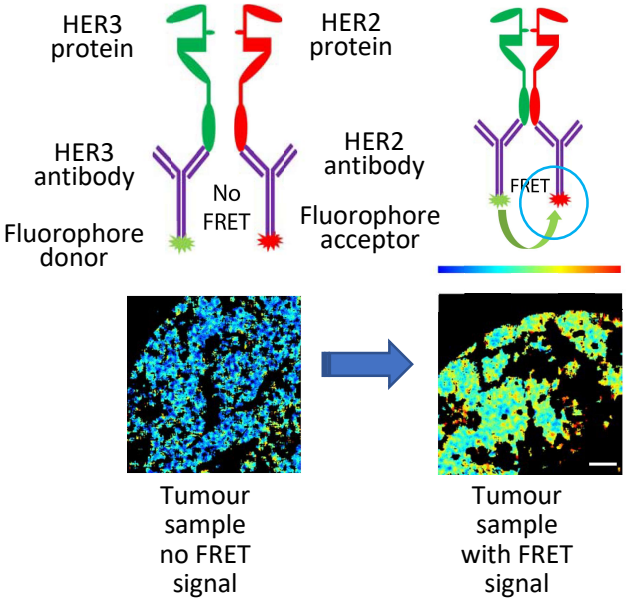
King's researchers have developed a novel imaging technique called Fluorescence Lifetime Imaging Microscopy Förster Resonance Energy Transfer (FLIM-FRET) for visualisation of complex protein-protein interactions in tissue samples from cancer patients.. FLIM-FRET has shown that certain protein combinations in tumour biopsies predicted either effectiveness or resistance to specific drugs. Thus, FLIM-FRET can help target treatments to the right patients, resulting in reduced healthcare costs, reduced morbidity and improved survival. Drawn by this revolutionary technology, Pharma companies have funded FLIM-FRET projects to develop and evaluate novel cancer drugs and a spin-out company, Nano Clinical, was created to further develop and commercialise this technique.

2. Underpinning research

Patients need to be matched to treatments most likely to target their particular cancer. Recent advances in cancer treatment target specific tumour types but only work when the right patients – those with the target tumour type – can be identified. King's research allows the revolutionary FLIM-FRET technology to visualize receptor pairings, increasing our understanding of tumour biology and leading to improved matching of treatments based on patient's specific tumour biology. Specifically, FLIM-FRET has been applied to visualizing epidermal growth factor receptors (EGFR) which are members of a family of proteins that play a key role in uncontrolled cell growth and are overexpressed or mutated in numerous cancers. Members of the EGFR family include HER1 (EGFR), HER2, HER3 and HER4. It is known that when two receptors within the EGFR family, such as HER2 and HER3, are proximally close (called dimerisation), it leads to enhanced tumour growth and the presence of this dimerisation may further predict success or failure of a particular therapy. For example, cancer patients with HER2-receptor positive cancer have been successfully targeted by drugs such as trastuzumab. Yet, these HER2 targeted treatments are still only 15-30% effective in colorectal and breast cancers. Further studies now show that proximity of HER3 to the HER2 receptors may induce drug resistance and suggest the addition of a drug which disrupts HER dimer formation, such as pertuzumab would improve outcome in these patients. Though this heterogeneity of EGFR protein interactions within tumour tissues has been proposed as a major mechanism underlying treatment failure for patients with cancer, there were no methods to analyse this phenomenon with existing light microscopes. Thus, better imaging methods were required to be able to apply this technique to clinical samples.

Better imaging methods are required to analyse clinical samples. Traditional light microscopes, coupled with immunocytochemical methods, allow visualisation of receptors by attaching a fluorescing molecule called a fluorophore to an antibody targeting a tumour protein of interest. However, the resolution limits of standard microscopes are limited by the wavelengths of light to about 250 nanometres (nm). However, to understand and visualize protein dimerisations requires resolutions <10 nm, roughly the size of the proteins themselves. This resolution can be obtained via an existing technique known as FLIM-FRET, which measures protein separations though a quantum interaction between fluorophores.

In FRET, one protein is tagged with a donor fluorophore and the other protein is tagged with an acceptor fluorophore. When the proteins are close enough to each other, the energy from an excited donor is transferred to the acceptor fluorophore which then fluoresces in a different colour. FLIM measures the decay rate of the fluorescence and hence the transfer rate. The closer the receptors are to each other the faster the transfer rate between them. Combining the two techniques in FLIM-FRET it is possible to visualize and quantify both the density of receptors and proximity of the dimer pairs to each other even at distances <10 nm (see adjacent figure).



King's investigated the potential of FLIM-FRET to better analyse how proteins are organised in tumour samples. In 2001, King's researchers began using FLIM-FRET microscopes they had built to analyse specific protein-protein interactions within tumours. While these instruments were able to identify EGFR interactions, the approach was too slow, too complex, too costly and lacked robustness for analysing a high-volume of samples required for clinical diagnostic applications. To overcome these limitations, King's developed new microscopes and analysis methods allowing high throughput, accurate assessments of *in vitro* and clinical samples **[1,2]**.

King's developed a new microscope and method of analysis to more accurately assess a greater number of clinical samples. Through successive prototyping, the latest FLIM-FRET system, called SWARM (SWept ARray scanning Microscope), was completed in 2019. SWARM replaced the single scanning beam with 1024 beams for both excitation and detection. This arrayed approach provided up to a 1024x speed improvement. This faster imaging time permitted an increase in the spatial resolution and in the sample acquisition rate. Additional features of SWARM reduced the manual, error-prone processes, and added algorithms for the detection of patient subgroups with different probabilities of survival and treatment outcomes. In addition to the SWARM microscope, a key element for application of this technology was the development of appropriate assays. This consists of identifying receptors of biological interest, developing targeted antibodies, and attaching a fluorophore. Applying the improved FLIM-FRET microscope to clinical cancer samples and creating the assays, King's discovered and validated EGFR dimerization pairs that inferred both drug resistance and drug effectiveness. These include HER2:HER3, EGFR:HER3, and EGFR:HGFR (hepatocyte growth factor receptor) applied to breast, colorectal, lung, and head and neck squamous cell carcinoma (HNSCC) cancers **[1-7]**. These are detailed in Section 4 below along with the clinical outcomes.

3. References to the research (indicative maximum of six references)

1. Tao et al., "Antagonism of EGFR and HER3 Enhances the Response to Inhibitors of the PI3K-Akt Pathway in Triple-Negative Breast Cancer," Sci. Signal., vol. 7, no. 318, 2014, doi: 10.1126/scisignal.2005125.
2. Ortiz-Zapater et al., "MET-EGFR dimerization in lung adenocarcinoma is dependent on EGFR mutations and altered by MET kinase inhibition," PLoS One, vol. 12, no. 1; 2017, doi: 10.1371/journal.pone.0170798.
3. Weitsman et al., "HER2-HER3 dimer quantification by FLIM-FRET predicts breast cancer metastatic relapse independently of HER2 IHC status," Oncotarget, vol. 7, no. 32, 2016, doi: 10.18632/oncotarget.9963.

4. Ng et al., “The use of exosome and immune profiling to analyze a phase 2 study on the addition of patritumab or placebo to cetuximab and a platinum agent for recurrent /metastatic head and neck cancer patients.,” J. Clin. Oncol., vol. 36, no. 15 May 2018, doi: 10.1200/JCO.2018.36.15_suppl.6043.
5. Claus et al. (2018) Inhibitor-induced HER2-HER3 heterodimerisation promotes proliferation through a novel dimer interface'. eLife vol7, doi10.7554/eLife.32271
6. Barber et al., “HER2-HER3 Heterodimer Quantification by FLIM-FRET and Patient Subclass Analysis of the COIN Colorectal Trial,” JNCI J. Natl. Cancer Inst., Dec. 2019, doi: 10.1093/jnci/djz231.
7. Li et al (2020) HER2-Mediated Internalization of Cytotoxic Agents in ERBB2 Amplified or Mutant Lung Cancers. Cancer Discovery vol10, doi 10.1158/2159-8290.CD-20-0215

4. Details of the impact

Getting the right drug to the right patient allows better outcomes to be achieved sooner, and means morbidity and suffering from the side effects from ineffective drugs would be eliminated. In the UK, an annual total of 156,000 patients have breast, HNSCC, lung and colorectal cancers of which 20% or (31,200) would likely benefit from targeted treatment and evaluation by FLIM-FRET. The research developed by King’s in the SWARM system has sped up improvements, targeted assays and validation studies that have made this technology viable for drug development and clinic application as demonstrated by the collaboration agreements with multiple drug companies, formation of a spinout company and early clinical trial results.

High profile drug companies have paid over GBP1,200,000 through collaboration agreements and are using FLIM-FRET to actively develop and test both novel and existing therapeutics in new cancer types. Pharma companies have funded a number of pre-clinical and clinical projects to develop the use of FLIM-FRET to better understand protein dimerisation and the impact on treatment effectiveness or resistance. This has allowed them to develop drugs targeted to prevent dimerisation and identify subsets of patients who will respond to their drugs:

Daiichi Sankyo is a global pharmaceutical company based in Japan that has a presence in more than 20 countries around the world. They have been working with King‘s for the past seven years on three separate drug development projects including a phase 2 trial with Patritumab/Cetuximab Combination Therapy in head and neck squamous cell carcinoma **[A]**. King’s analysis as part of this trial provided Daiichi Sankyo with data on how and why a subgroup of patients reacted positively to their drug. The senior Director for global Oncology R&D said **[A]** “*The technology is very ground-breaking, very scientifically sound, advanced and creative. This technique allows us to better understand the immune system and the drug related outcomes of patient*”.

Roche Products Ltd is a Swiss multinational and the world's largest biotech company, with differentiated medicines in oncology, immunology, infectious diseases, and neuroscience. As a result of King’s EGFR-HER3 dimer development programme, in 2016 Roche took an interest in the technology and funded a study at King’s that used FLIM-FRET to validate HER2-HER3 dimerisation **[B]**. The study demonstrated HER2:HER3 dimerization was significantly associated with survival independent of HER2 expression **[3; D]**. These findings indicate that the quantification of level of HER2-HER3 dimers could guide the stratification of patients with breast cancer towards HER2-directed therapies. Based on these results and with additional funding from Guy’s Cancer Charity, a pilot study to measure the HER2-HER2 dimer expression in breast cancer patients receiving HER2 targeted therapies (NCT04288141) was initiated and recruitment opened from January 2020, which was paused due to COVID-19 but has now recommenced.

AstraZeneca, one of the largest pharmaceutical companies in the world, has funded a pre-clinical study at King’s to investigate FLIM-FRET as part of a predictive biomarker study for the EGFR inhibitor AZD9291 (Osimertinib) **[C]**. This led to a Cancer Research UK Multidisciplinary Project Award entitled “Multimodal advanced imaging to probe plasma exosomes for EGFR network re-

wiring: A potential treatment resistance mechanism in lung cancers" (C7674/A21840). The results of these studies are now in a pre-submission manuscript entitled "Combined osimertinib and anti-HER3 treatment engages immune-dependent tumour toxicity by macrophages in non-small cell lung cancers."

UCB Pharma is a global biopharmaceutical company headquartered in Belgium, with a focus on neurology and immunology. The company is a top 5 investor in biopharmaceutical R&D in the UK. In 2019, UCB selected King's as a partner in testing their new anti-cancer drug UCB6114 (ClinicalTrials.gov Identifier: NCT04393298). King's is leading a Phase I trial (2020-2023). The FLIM-FRET analysis directly contributed to UCB's decision to partner with King's; UCB have stated **[E]**: "*Looking at combination therapy will help to prioritize assets and translate this to clinic. Identifying the best combinations is important and this technology will help us define this".*

Incyte is an American pharmaceutical company founded in 1991 and based in Delaware. In 2019 it had a revenue of GBP1,680,000,000. They have recently selected King's to partner with to use FLIM-FRET to study dimerization of another cancer-associated receptor PD-L1 **[F]**. This study commenced in late 2020 and will reveal the mechanism of action of Incyte's lead anti-PD-L1 compound.

The promise of clinical applications of FLIM-FRET has led to formation of a spinout company, Nano Clinical Ltd. With the proof-of-concept now well established, the key to unlocking the full impact will be to obtain regulatory approval, conduct definitive validation studies and make FLIM-FRET widely available. To this end, a patent was filed in 2017 (subsequently extended to EU; **[G]**) and a company was established in 2019 (**Nano Clinical Ltd**.); a CEO has been recruited to begin the fund-raising process. It has already attracted a team of seasoned entrepreneurs and scientists willing to invest their own time (~3 fulltime equivalent years) and money (~GBP200,000) to build a company and services around FLIM-FRET. The CEO has stated **[H]**: "*This appealing technology is a "missing link" providing unique functional information that explains the biological mechanism of how drugs work. There are no comparable technologies capable of providing this critical piece of biological information. The initial fundraising goal is a seed fund of GBP1-2,000,000; with an initial GBP500,000 tranche in which I will personally contribute about half. This funding, in addition to revenue coming from our service business to Pharmaceutical companies should enable us to employ ~5 persons in the first year, and ramp-up to about 80 in the fifth year".* Nano Clinical are refining FLIM-FRET technology for more widespread clinical use and aim to seek FDA approval in 2021 for siting instruments into clinics worldwide for diagnostic purposes.

Early clinical studies validated the technique and demonstrated the impact of FLIM-FRET to cancer patients. In a phase II clinical trial in HNSCC sponsored by Daiichi Sankyo (NCT02633800, 2015-19), identifying EGFR:HER3 dimers from head and neck cancer patient serum exosomes was predictive of the treatment response to patritumab within 3 weeks, after a single treatment cycle **[4]**.

In lung cancer, patients with high levels of EGFR:HGFR dimerization in tumours do not respond to EGFR therapies. However, FLIM-FRET analysis has demonstrated *in vitro* and *in vivo* that tumours with high EGFR:HGFR dimers were more likely to respond to HGFR targeted therapy, providing more powerful means to personalise therapy compared to the traditional approach of analysing overall levels of HGFR **[2]**. One of the challenges of targeted, personalised therapy is identifying the correct patients who will benefit. In a phase 3 colorectal trial (NCT00182715, 2000-2003) there was no significant benefit to globally adding cetuximab to oxaliplatin based therapy. Retrospective analysis of samples from this trial using FLIM-FRET was able to identify a sub-group of ~10% of the colorectal cancer patients with lower HER2:HER3 dimers that showed significant clinical improvement in response to this combination therapy. 44/398 patients who were identified as benefiting from having EGFR-targeted therapy added to standard chemotherapy had a significantly longer progression-free overall survival (difference = 221 days, median, 21 months

[6]. This demonstrates the application of FLIM-FRET to identify patients with HER2-HER3 dimers provides previously unappreciated insight into those who benefit from this treatment.

Breast cancer patient biopsy samples analysed by FLIM-FRET showed EGFR-HER3 and HER2:HER3 dimers predicted treatment resistance and likelihood of metastatic relapse [1, 3]. Further pre-publication data from the team found that HER2-HER3 dimerisation may render HER2+ breast cancer patients less responsive to HER2-targeted treatment trastzumab. Based on this study, an oncologist at Memorial Sloan Kettering (MSK) worked with the King's team to determine whether this effect would also be seen in HER2-positive lung cancer patients. Their findings demonstrated that HER2 inhibitors were further enhanced by co-treatment with a HER2-HER3 dimerisation inhibitor, resulting in improved patient outcome [7] The MSK oncologist stated **[I]**: *"We have been investigating the use of antibody drug conjugates (ADCs) for the treatment of cancer with HER2 alterations. FLIM allowed us to determine that the responders to ADCs had HER2-HER3 dimerization, providing a potential mechanistic explanation for the entry of ADCs into tumours without HER2 expression (…) FLIM technology provides us with a tool to gain better mechanistic understanding of ADC response as several pharma companies are focusing their clinical development of ADCs to targeting HER2 mutations with active dimers (…) FLIM has helped motivate a shift in mindset for both investigators and funding agencies such as NIH based on our preliminary data and the ability to analyse trial data to gain mechanistic insights. With this change in direction of research funding and interest by pharma, FLIM has opened up potential new areas of promising research for cancer patients."*

Additionally, T-DM1 treatments are now part of the National Cancer Comprehensive Network (NCCN) clinical guidelines for treating HER2 mutated lung cancers based on the FLIM-FRET data reported in **[J]** and thus currently impacting patient care **[K]**. These guidelines **[K]** detail the sequential management decisions and interventions that currently apply to 97% of cancers affecting patients in the United States. They are the recognised standard for clinical direction and policy in cancer care.

5. Sources to corroborate the impact

A. Testimonial from Senior Director, Global Oncology R&D, Daiichi Sankyo, Inc.

B. [text removed for publication]

C. [text removed for publication]

D. Cancer Research UK press release, Imaging technique could help focus breast cancer treatment, 07/07/2016 - https://www.cancerresearchuk.org/about-us/cancer-news/press-release/2016-07-07-imaging-technique-could-help-focus-breast-cancer-treatment

E. Testimonial from Satellite/Hub UCB-KCL Head, UCB Pharmaceuticals

F. [text removed for publication]

G. Next generation of FLIM systems UK patent- Luminescence Imaging Apparatus and Methods, Application no: 1710743.4 (Submitted July 2017, since extended to EU)

H. Testimonial from CEO, Nano Clinical LTD.

I. Testimonial from Consultant Medical Oncologist, Thoracic Oncology and Early Drug Development Service, Division of Solid Tumor Oncology, Department of Medicine, Memorial Sloan Kettering Cancer Center.

J. Li et al, IASCL 19th World Conference on Lung Cancer, 2018 P1.13-43

K. NCCN Flash eBulletin Newsletter Update: NCCN Guidelines, NCCN Compendium NCCN Templates & NCCN Radiation Therapy Compendium for NSCLC