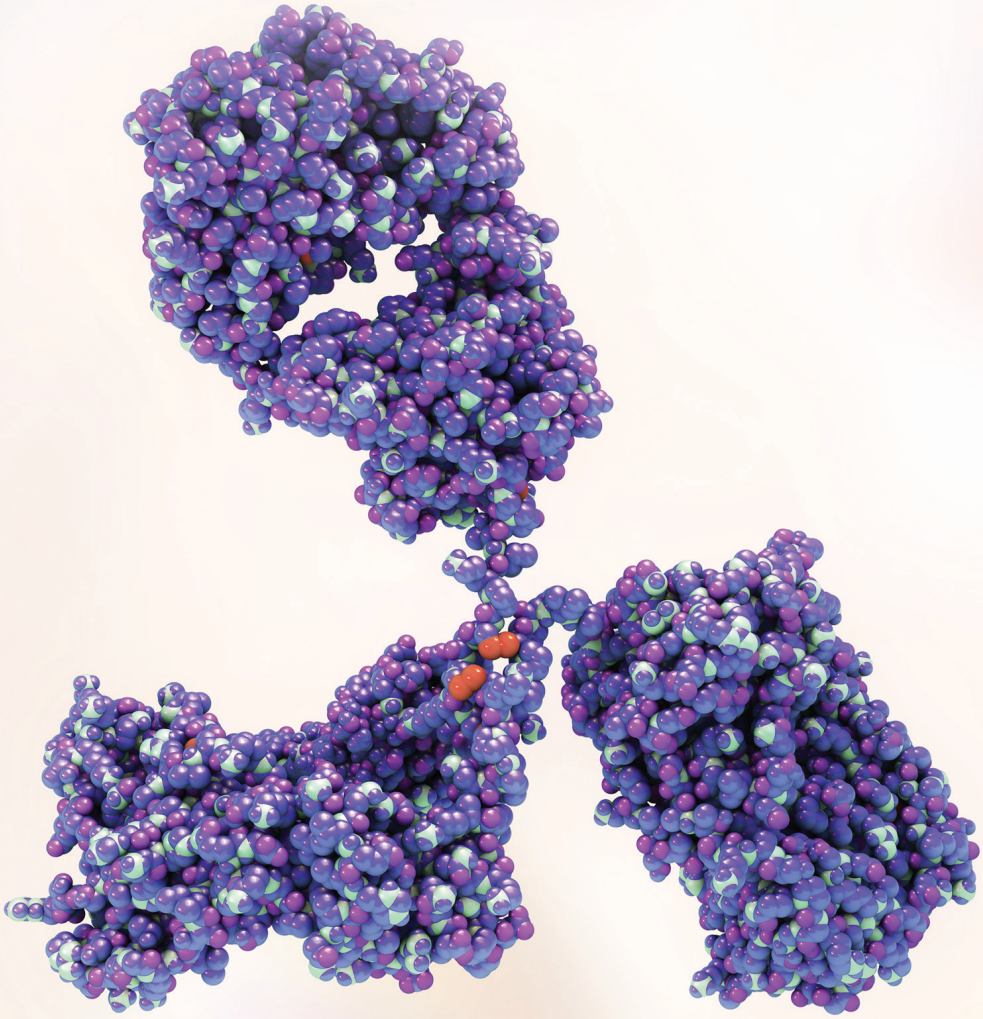
Syngene

Putting Science to Work

Antibody Drug Conjugates

Re-emergence of an old modality for targeted therapies



Introduction

Antibody drug conjugates (ADCs) are a class of targeted drugs composed of a cytotoxic payload linked to an antibody that targets a cancer cell, designed to release the payload specifically at a tumor site to kill the tumor. ADCs combine the selectivity of antibodies with the efficacy of small molecule drugs, allowing for more precise, targeted therapeutic applications.

This modality is also being explored using non-toxic payloads for immune modulation, including for indications such as autoimmune disorders. Other modalities include antibody-PROTACs for targeted degradation and antibody-oligo conjugates. As of March 2024, there are 15 FDA-approved ADCs in the market and about 300 in clinical trials.

In this White Paper, we explore the key challenges in ADC development and how Syngene, a leading CRO/CDMO, supports clients in the discovery, development, and manufacturing of innovative ADC molecules.

Industry outlook for ADCs

Market research data reveals a robust growth trajectory for ADCs. According to Evaluate Pharma, sales for the 11 ADCs currently on the market are expected to expand from $6.8 billion in 2022 to $19.9 billion in 2027, representing a remarkable five-year compound annual growth rate (CAGR) of 24.0%.[1] A new analysis by Roots Analysis has predicted that the antibody-drug conjugates (ADC) market will be worth more than $15 billion by 2030, growing at a compound annual growth rate (CAGR) of over 20 % due to their growing popularity and therapeutic potential.[2]

The antibody-drug conjugates technology market size in the next-generation antibody market for North America was worth US$ 1,246.93 million in 2021. The U.S. market held the highest share with revenue of US$ 1,055.06 million in 2021 and is expected to grow at a CAGR of 12.0% during the forecast period[3] (2021-28). All these market surveys point towards a remarkable 15-24 % growth in the next few years, making ADCs one of the promising therapeutic areas.

Challenges in ADC discovery

ADC discovery involves a multidisciplinary approach that combines knowledge from antibody engineering, linker chemistry, and payload selection. Further, the discovery, development, and manufacturing of ADCs present numerous challenges due to the complexity of these molecules and the need to balance efficacy, safety, and manufacturability. The key challenges in ADC development process are as follows:

Payload selection and optimization: Selecting cytotoxic payloads with potent anti-tumour activity and favourable pharmacokinetic properties while minimizing off-target effects and systemic toxicity.

Linker design: Designing linkers that are stable in circulation but cleavable in the tumour microenvironment to release the cytotoxic payload selectively.

ADC conjugation: Achieving selective conjugation of the cytotoxic payload to the antibody while preserving the structural and functional integrity of both components is crucial. Non-specific conjugation can lead to heterogeneity in the final product, affecting efficacy and safety.

Purification challenges: Overcoming challenges associated with purifying heterogeneous mixtures of ADCs while removing impurities and maintaining product stability.

ADC discovery and development at Syngene

At Syngene, we have nearly all the capabilities required to execute an end-to-end ADC program. In discovery, we offer the design and synthesis of linkers and payloads and support the identification of novel antibody binders, conjugations, and cell-based assays to demonstrate cytotoxicity. We also undertake *in vivo* proof-of-principle studies in animal models combined with PK studies and toxicity studies. In development, we offer services in GMP manufacturing of antibodies, linkers, and payloads. We are planning to add GMP conjugation to our ADC offerings. We also have services to evaluate the efficacy and safety of ADCs in clinical trials.

Chemistry

Syngene's chemistry team has helped its clients design/modify novel payloads by providing novel structures for *in silico* evaluation and synthesizing the accepted molecules for *in vitro* evaluation. We have also optimized payloads based on their binding efficiency to target proteins measured via specific *in-vitro* functional assays.

Linker design is a key component of ADCs, primarily because of its impact on stability, solubility, and release of payloads. The linker choice allows for fine-tuning of DAR and PK of ADCs and reduction of aggregation.

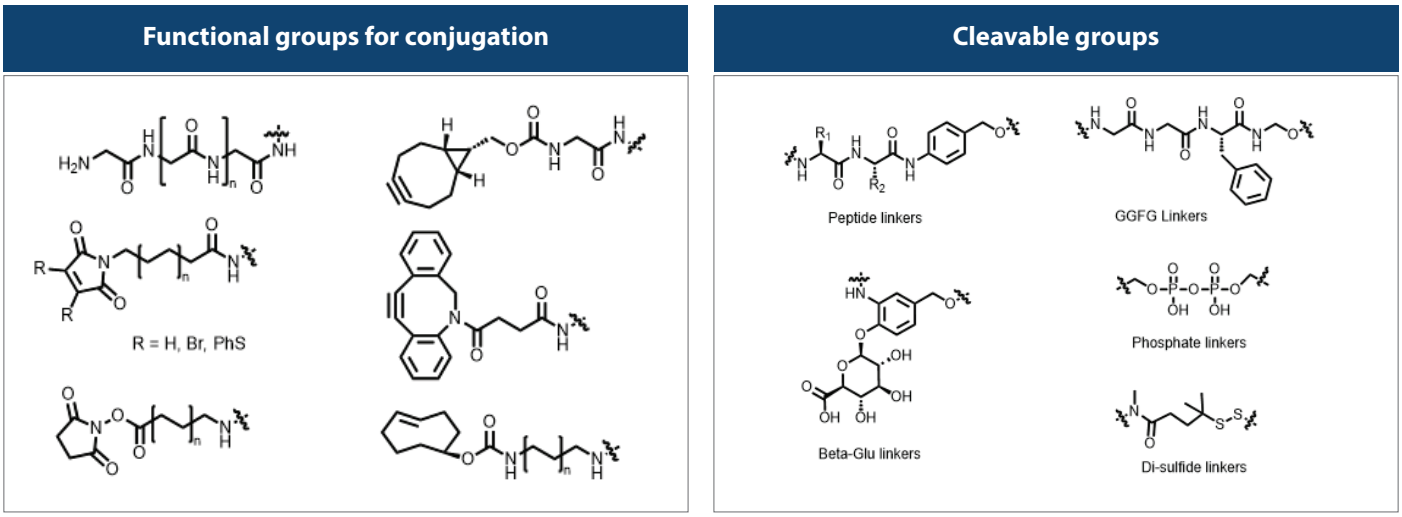


Figure 1: Various functional and cleavable groups used in ADC conjugation

Syngene has successfully designed a novel linker system and established proof-of-concept. It was patented by the sponsoring client **(WO 2015038426A1).**

Syngene scientists have also designed a novel process for preparing a class of high potent compounds, ***"Camptothecin derivatives"***, for one of their premier clients, which is suitable for large-scale manufacturing. It was patented by the sponsoring client **(WO 2024020734)**.

Biology

Our protein scientists make the immunogen and transfer it to the Antibody Discovery team, who identify the best binders using ELISA, SPR, or FACS by screening Ab libraries or using hybridoma, single-B cell cloning, or yeast display platforms. Our protein scientists scale up the antibodies recombinantly for conjugations. Amine or thiol conjugations utilize lysines and cysteines. Site-specific conjugations are performed by introducing a lone cysteine (Thiomab) or sites for enzymatic conjugations using transglutaminase. We also explore two-step conjugations using click chemistry and Diels-Alder chemistry for dual conjugations.

In addition to conjugating toxic and non-toxic payloads (for which we have dedicated isolators for safety), we also have experience in antibody-PROTAC and antibody-oligo conjugations. We can perform these at up to 100 mg scales, which can then be combined to get gram scales for preclinical and tox studies. Our rigorous analytical QC entails LC-MS and HIC for DAR ratios and homogeneity, SE-HPLC for aggregation and purity, and endotoxin levels. In addition, we are equipped to conduct accelerated stability studies at RT, 37°C and -80°C

Cell-based assays

Syngene has approximately 300 banked cancer cell lines from ATCC, with predetermined growth kinetics. By employing the Incucyte platform, we can determine the time course of cell kill in real-time. We have adopted this for 3D spheroidal cultures derived from the ATCC lines.

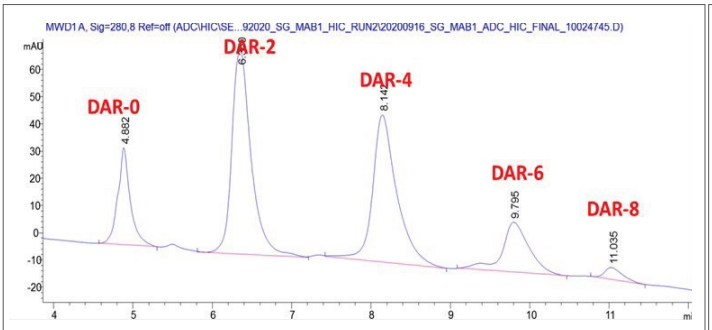


Figure 2: HIC-HPLC analysis of ADCs to determine DAR ratios

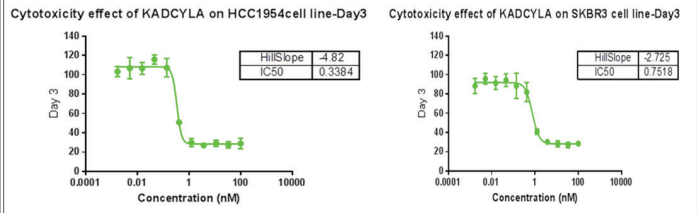


Figure 3: Cytotoxic effect of Kadcyla (a marketed Trastusumab ADC) on breast cancer cell lines

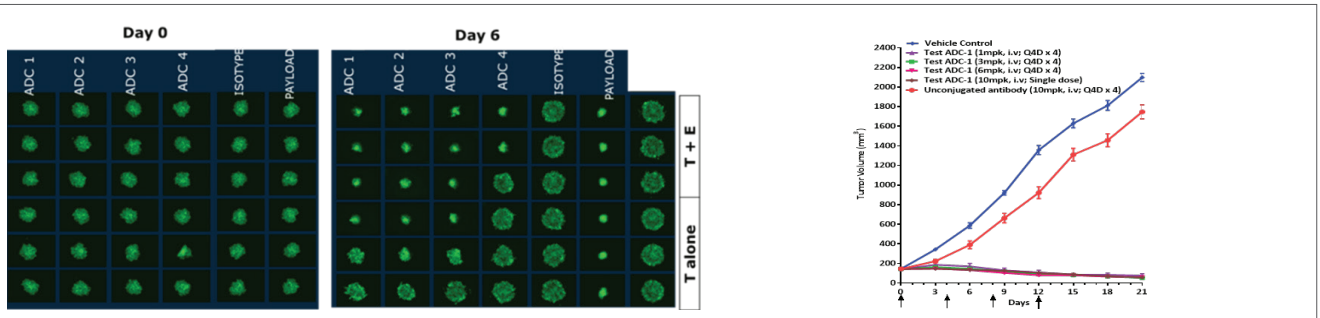


Figure 4: Left: Incucyte images of different immune-stimulating ADCs on the growth of 3D spheroidal cancer cultures in the presence of T cells. Right: In vivo efficacy of an ADC in a mouse xenograft model.

ADC conjugation and purification process[4]

Depending on the conjugation chemistry employed, we assemble various types of ADCs, such as cysteine-conjugated, lysine-conjugated, or site-specific ADCs. At this stage, it is crucial to recognize that the level of heterogeneity within the ADC is influenced by the conjugation strategy employed to link the drug to the antibody via the linker. All ADC molecules exhibit intricate chemical structures at a molecular level, combining the molecular characteristics of the linker and payload with the antibodies.

During the ADC process, the conjugation reaction employs a naked antibody and a high potent API (HPAPI) component. HPAPIs are classified as band 4/5 compounds due to their very high acute toxicity. Handling these substances requires stringent containment measures to protect both operators and the environment within the manufacturing suite. Even minute quantities of HPAPIs can have a significant biological effect, making containment a top priority. The HPAPIs used in ADCs often include microtubule-disrupting agents and DNA-modifying agents, which are highly effective in targeting both dividing and non-dividing cells.

The HPAPI complex with the antibody is conducted in an isolator suite. We employ bulk ultrafiltration or chromatography to remove process contaminants such as free cytotoxin and organic solvent. The steps involved in a typical ADC conjugation and purification process are described below (Figure 5).

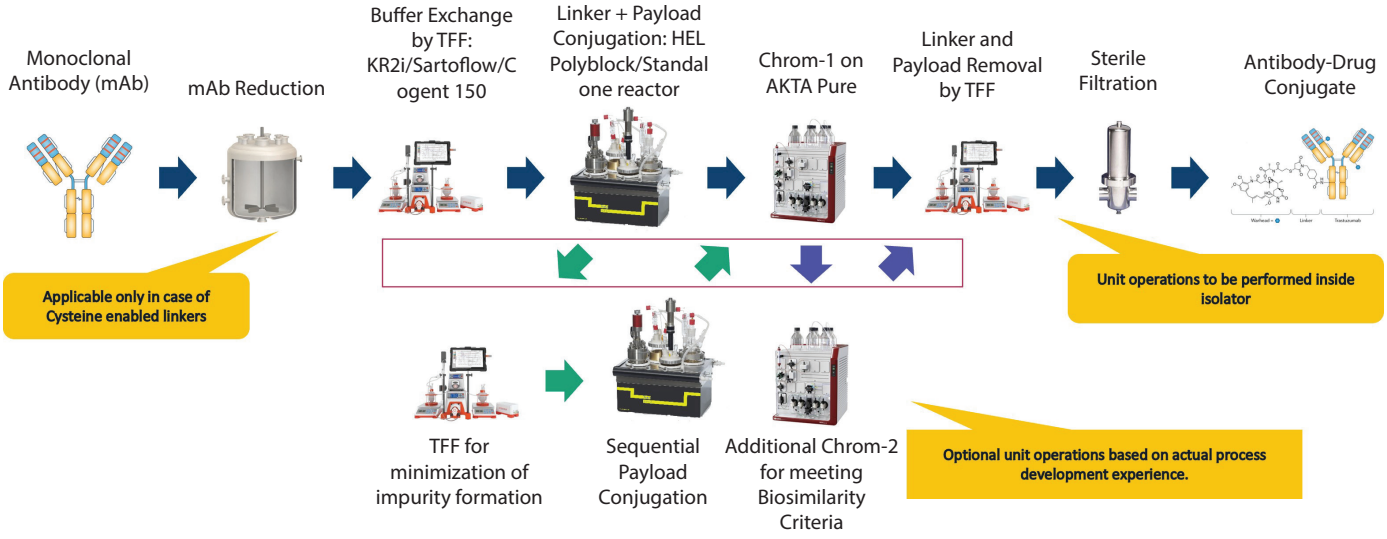


Figure 5: ADC conjugation and purification process

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Syngene's experience with client programs

Syngene's Discovery Chemistry and Discovery Biology teams have successfully addressed the issue of premature payload release in ADCs, minimizing side effects like hematotoxicity, hepatotoxicity, and gastrointestinal reactions. They have developed next-generation ADCs with improved release kinetics and properties, enhancing the therapeutic index. Syngene focuses on increasing the stability of ADC molecules in circulation by employing novel linker chemistry and a site-specific conjugation strategy. This approach ensures the Development of homogeneous ADCs with improved properties.

Syngene's collaborative efforts with clients have led to developing a first-in-class ADC currently undergoing Phase I trials for advanced solid tumors. The improved dosing accuracy and enhanced safety profile of this ADC positions it as a superior alternative to conventional ADCs. It can synthesize and handle highly potent active pharmaceutical ingredients (HPAPIs) in various quantities, ranging from a few milligrams to grams in a development facility and up to 30 kilos in a single batch under cGMP.

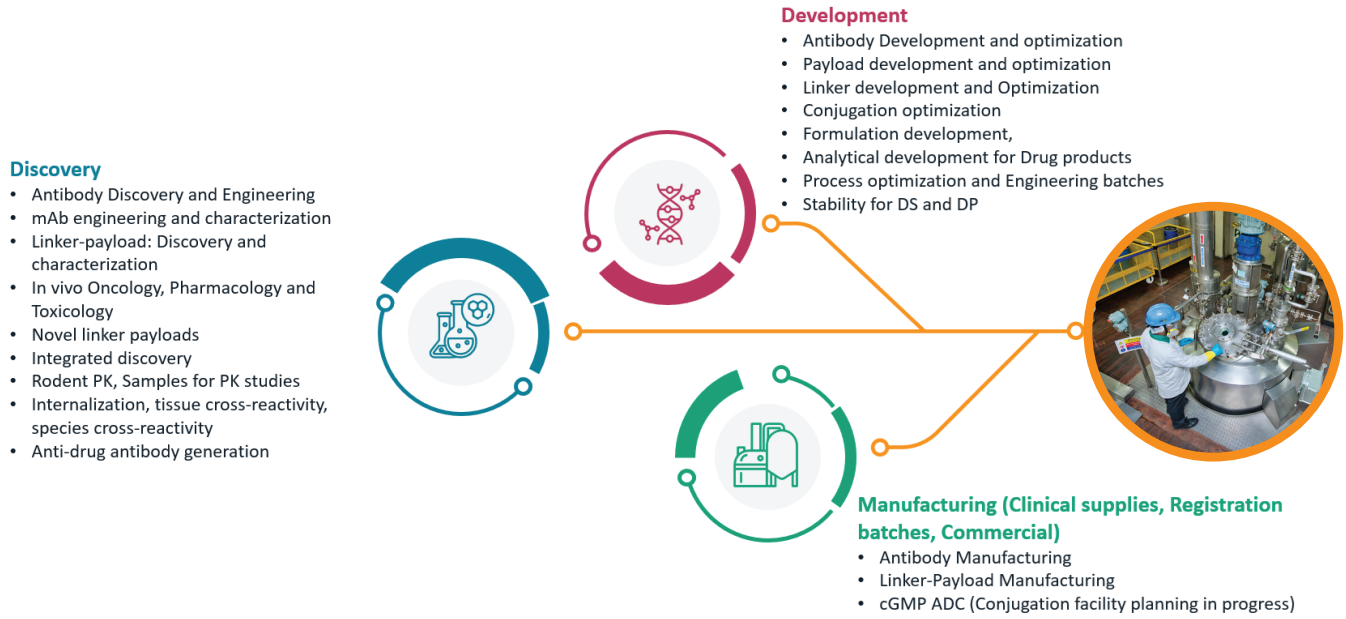

Figure 6: Syngene's experience in ADC

Our ADC track record

Syngene has excelled in delivering ADC projects during the discovery phase, intricately crafting linkers, payloads, and antibodies, followed by meticulous optimization to meet client project requirements. We are embarking on establishing an advanced ADC conjugation facility, poised to bolster development capabilities and facilitate cGMP scale production.

Our chemical development and biologics operational units converge synergistically, with their wealth of knowledge and experience, while building infrastructure to advance ADC in developmental and cGMP scales.

ADC components projects completed for ADC	Antibody for ADC		Cytotoxic drugs		Linker		cGMP ADC conjugation*	
	nGMP	cGMP	nGMP	cGMP	cGMP	cGMP	cGMP	cGMP
	>10 (discovery scale)	1	>120 (discovery scale)	3	>70 (discovery and development scale)	4	>15 (discovery scale)	Capability development is in progress

***Setting up development and scaleup facility is in progress**

Table 1: Projects delivered at each phase of the ADC service line

Summary

The development of ADCs has opened new avenues in the treatment landscape, particularly for tumours that were previously difficult to address. As research continues to refine ADC design, including optimizing linker chemistry and exploring novel payloads (including non-toxic payloads), the promise of even greater precision and potency in targeting tumours using improved technology looks imminent. Furthermore, ongoing efforts to enhance manufacturing processes and address challenges such as drug resistance can potentially expand the utility of ADCs across a broader spectrum of malignancies.

At Syngene, we have most of the capabilities required to execute an end-to-end ADC program, from discovery to manufacturing. We also have the services to evaluate the efficacy and safety of ADCs in clinical trials.

To know more about our ADC services, **contact our experts**

About the author

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VP and Head, Discovery Biology, Syngene International Ltd.

Jayashree Aiyar holds a Ph.D. in Immunology from the All India Institute of Medical Sciences, New Delhi, and has pursued her post-doctoral research at the California Institute of Technology and the University of California at Irvine.

She has over 20 years of global experience as a molecular pharmacologist with leading global organizations like AstraZeneca, Merck, Ambrx, and Theravance.

She has more than 30 peer-reviewed publications and book chapters to her credit.

At Syngene, Dr Aiyar leads 700 scientists supporting small and large molecule drug discovery as well as cell and gene therapy.

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2. *https://www.europeanpharmaceuticalreview.com/news/98037/antibody-drug-conjugates-market-15bn-2030/*
3. *The INSIGHT partners, Antibody Drug Conjugates Technology Market Size in Next-generation Antibody Market, Global and Regional Analysis to 2028*
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About Syngene

Syngene International Ltd. (BSE: 539268, NSE: SYNGENE, ISIN: INE398R01022) is an integrated research, development, and manufacturing services company serving the global pharmaceutical, biotechnology, nutrition, animal health, consumer goods, and specialty chemical sectors. Syngene's more than 6000 scientists offer both skills and the capacity to deliver great science, robust data security, and quality manufacturing, at speed, to improve time-to-market and lower the cost of innovation. With a combination of dedicated research facilities for Amgen, Baxter, and Bristol-Myers Squibb as well as 2.2 Mn sq. ft of specialist discovery, development and manufacturing facilities, Syngene works with biotech companies pursuing leading-edge science as well as multinationals, including GSK, Zoetis and Merck KGaA.

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