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Challenges and opportunities of next generation therapeutics: A compound management perspective

Silvio Di Castro *, Martin L. Svensson

Compound Synthesis & Management, Discovery Sciences, R&D, AstraZeneca, Gothenburg, Sweden

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ABSTRACT

In recent years, pharmaceutical research has increased its interest in novel drug modalities beyond small molecules to overcome therapeutic limitations and find more effective cures.

Compound Management teams globally are adapting their processes and equipment to handle such "new modalities" with the same quality and speed as small molecule research.

Here, we share our approach in supporting next-generation therapeutics by introducing solutions for multi-solvent workflows in Compound Management at AstraZeneca.

From sample submission to assay-ready plate generation, we describe the challenges faced and process improvements introduced so far.

Collaborating with our business partners, we are pioneering new best practices and laying solid foundations for future research to bring new efficacious drugs into the clinics.

1. Introduction

The pharmaceutical industry is undergoing a significant transformation driven by the emergence of next-generation therapeutics [1–4]. These "new modalities" can benefit patients by treating new diseases, providing more effective, convenient, or personalized treatment, or complementing existing therapies.

As a major player in the world of biopharmaceuticals, AstraZeneca is investing significantly to introduce non-small molecule research (peptides [3], targeted protein degradation therapeutics [5], oligonucleotides [6]) to the company's portfolio.

The initial scientific evaluation of a new modality usually begins with exploratory work led by internal project teams or through external collaborations. Over time, the number of samples and experimental data might grow beyond what can be managed by researchers through spreadsheets and manual bookkeeping. Usually, this is where Compound Management (CM) provides help and expertise.

Also known in the industry as Sample Management or Sample Logistics, the role of any CM team is key to early pharmaceutical research. Tasked with curating the quality of both samples and their data, CM ensures samples are available for scientists in a variety of ready-to-use formats, providing consistency and traceability from submission to experiment and data analysis.

While effective for small molecules, the existing tools and workflows are often inadequate to address the complex landscape of new modalities. We need novel solutions to strike a new balance between flexibility and productivity and to process multiple modalities in the desired format and solvents while retaining operational excellence.

2. Challenges of multi-modality workflows

CM has traditionally relied on organic solvents, typically dimethyl sulfoxide (DMSO), for dissolution and screening of small molecules. Over decades, the industry developed and adopted best practices to establish productive, streamlined, and automated workflows for the management of millions of small molecules [7–9].

Working with new molecule types and solvents with different physical properties and requirements demanded rethinking of existing processes. We adapted our processes in the short term, while striving to create future-proof solutions based on the new requirements.

We encountered challenges, either related to intrinsic properties of the materials involved or to the data needed to uniquely identify the material and the desired workflow. Table 1 shows common questions that need answering for a successful implementation of a new modality workflow.

While some answers can be found in literature, novel studies are

* Corresponding author.
E-mail address: Silvio.DiCastro@Astrazeneca.com (S. Di Castro).

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Table 1
Examples of challenges and questions that need addressing when a new modality enters the scope of CM operations.

Solvent type	Which solvents are compatible with the modality in question? Is more than one option applicable? Are there any exceptions and how can these be detected?
Process environmental control	Is the process compatible with standard laboratory air? Is humidity and/or temperature control required?
Storage temperature	What is the storage temperature for samples not in solution? What is the storage temperature for solubilized samples? Is there a difference between short- and long-term storage temperatures?
Concentration	Can nominal concentration be assumed as adequate when a solution is created, or should it be measured? Is concentration normalization required to account for differences in synthesis yield?
Water absorption or evaporation	Is the solvent hygroscopic? Is the solvent likely to evaporate significantly during sample processing and/or storage?
Chemical stability	How is chemical stability affected by storage conditions, solvent, freeze/thaw?
Sample registration parameters	How to discern which processes are allowed or possible? Can we use registration information to uniquely define and steer the workflow?
Information management systems	Are existing IT systems and tools compatible with the required registration parameters? Do we need new IT solutions to support or enhance the existing tools?

often required to gather additional information to ensure the quality of the new samples. Similarly, off-the-shelf IT (Information Technology) solutions are seldom available, thus driving the development of bespoke tools. As knowledge builds up, CM can support scientific research while evaluating and developing more robust solutions for the future.

3. Compound management process adaptation

AstraZeneca follows an established CM process framework represented in Fig. 1. Any changes to accommodate new modalities are captured and incorporated in the relevant steps. Quality assurance methods underpin the entire process and are employed regularly to maintain the quality of each step.

3.1. Registration

Registration is the process that creates a new molecular entity in the company's database, characterizing it univocally. Rather than rewrite the legacy corporate registration system for small molecules, we complemented it with a new one (RegMol, Scilligence, Cambridge, MA).

Peptides and oligonucleotides are the first modalities supported with new fields and descriptors (e.g., oligonucleotide sequence, peptide sequence). We added generic descriptors (e.g., sample type) to clarify the nature of the material and simplify the downstream workflows. These descriptors will also help us to extend the scope in the future and include further modalities.

3.2. Submission

Submission is the step of physically handing over materials to CM, and their data association to a container (tube, vial, microtiter plate). The submission format may vary depending on the desired workflow.

We developed informatics tools to capture new parameters (e.g., solubilization instructions, solvent required, final concentration, storage temperatures) along with basic information (e.g., labware barcode, sample amount, unique identification number).

This helped us shift from the world of "unstated defaults" of small molecules (e.g., with DMSO being the only suitable solvent for the screening collection, there was no need to capture any information about "solvent required") to highly detailed process instructions that minimize risks of mishandling after submission. All new parameters are passed from the submission tools to the LIMS (Laboratory Information Management System) used by CM (Mosaic, Titian Software, UK).

Individual samples ("singletons") are submitted either insolubilized ("neat") in pre-tared vials or as a liquid solution of known amount and concentration in automation-friendly screwcap tubes. Sample collections ("libraries") are submitted as lyophilized powders in deep-well microtiter plates (96-well 2 mL deep-well).

The amount submitted can vary (e.g., based on purpose and stage of the project, type of modality, complexity of chemical synthesis). Project prioritization is required if the amount is limited and insufficient to cover all the standard research activities. At present, prioritization via LIMS is not enabled, but workarounds are in place (e.g., verbal or written communication, color-coding).

Process dead volumes may occasionally be a constraint when limited amounts of compound are synthesized, thus requiring special agreements with the sample supplier. For instance, a neat sample requires solubilization to a given concentration before it enters the screening collection. This process consumes more material and increases the processing time. However, submitting the sample already solubilized at the right concentration and in the right labware speeds up the process and minimizes sample losses.

3.3. Sample preparation

Following submission, CM performs any mandatory preparation steps before submitted materials become available to researchers. The sample preparation steps depend on the modality type, on the submission labware type (plate or vial), and on the form of the material submitted (neat sample or solution).

The diagram in Fig. 2 provides an overview of the typical steps that follow submission. To enable higher throughputs at the delivery stage, we dissolve powders to generate solutions at a workflow-driven concentration (e.g., 0.1 mM, 0.5 mM, 1 mM). Such solutions are then

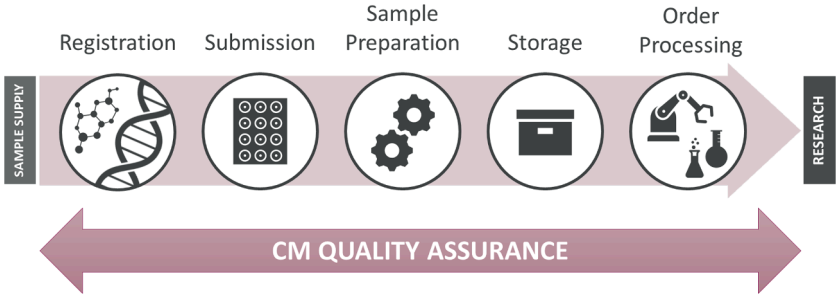

Fig. 1. Overview of the Compound Management process, outlining the steps that enable any modalities to enter CM and become available for researchers and their experiments. Quality assurance methods are applied throughout as required.

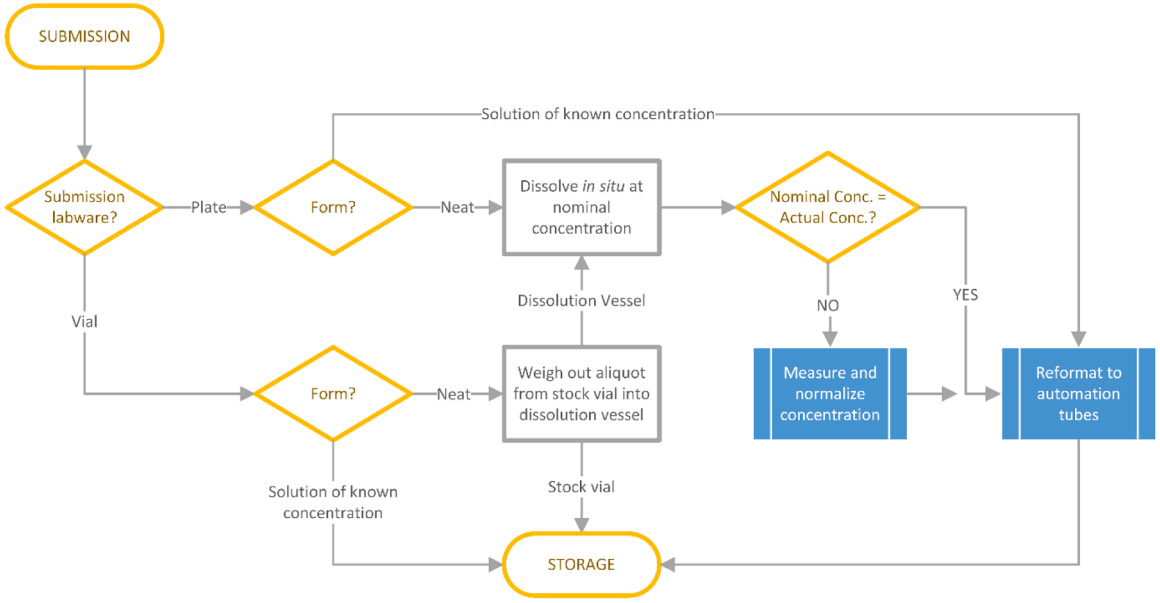

Fig. 2. Sample preparation workflow overview. To streamline the workflow, CM requires any submitted solutions to be at a known and agreed concentration, in automation-friendly tubes where applicable. This minimizes the sample processing steps and accelerates the availability of molecules to research projects. Exceptions are omitted from the diagram for simplicity.

transferred to automation-friendly tubes making them available for storing or order processing.

The submission of small molecules is governed by stringent purity criteria and—when solutions are generated from neat samples at a nominal concentration—their actual and nominal concentration are assumed to be equal. The same cannot be said for other modalities, for example oligonucleotides, for which the synthetic yield is unknown at the time of submission. We developed methods and IT tools to measure and normalize the concentration of oligonucleotide solutions; more details can be found in Section 4.

3.4. Storage

After preparation, samples (liquid solutions in tubes; neat samples in vials) are stored at conditions appropriate for the molecular stability of the compounds and solvents.

3.4.1. Neat samples

Neat samples are stored in environmental cabinets (21 °C, 10 % RH), refrigerators (4 °C) or freezers (−20 °C) based on the storage requirements specified at submission.

3.4.2. Sample solutions

DMSO solutions are stored in tubes that are compatible with acoustic dispensing devices (Acoustic Sample Tube, Azenta Life Sciences, Burlington, MA). They are kept in an automated storage system (SampleStore III, Azenta Life Sciences) [7] in a controlled environment (21 °C, 10 % RH). When used with acoustic dispensing devices, acoustic tubes are surveyed, and their volume is verified as part of the transfer protocol within such devices [10]. LIMS records the survey volumes to keep accurate inventory information over time.

Aqueous solutions are instead stored in high-volume sample tubes (0.7 mL dual-coded tube with internal thread, Azenta Life Sciences). They are kept in an automated storage system (BioStore SE, Azenta Life Sciences) at −80 °C to avoid microbial growth during long-term storage and to minimize effects of evaporation. These high-volume tubes are not compatible with acoustic dispensing and surveying; thus, only nominal volumes are tracked in LIMS, which could lead to discrepancies over time. To increase the quality of our inventory data for high-volume tubes, we invested in a device (XL9, SPT Labtech, UK) for routine gravimetric verification and inventory update.

3.4.3. Storage temperatures

To minimize processing errors in CM, we introduced new mandatory submission parameters (neat storage temperature and solution storage temperatures). These help CM to maintain sample quality. For example, by checking these parameters, the store's control application rejects any attempts to store samples at the wrong temperature. Also, LIMS uses this information to steer the "CM delivery location" (benchtop, refrigerator, freezer) where the researchers will find the samples they requested once an order is complete.

3.5. Order processing

After any mandatory preparation steps, samples become available and researchers can order them via LIMS in the format most suited to their experiment.

3.5.1. Neat samples

To fulfil orders for neat samples, we manually weigh aliquots from the storage vials to a variety of destination vial options. The process takes place in a laminar airflow cabinet (ninoSAFE class II Cyto, Ninolab, Sweden) using a high-precision balance (Mettler-Toledo XPE205 DeltaRange, Mettler-Toledo, Switzerland), independently of the modality being weighed.

3.5.2. Sample solutions

To fulfil orders for samples in solution, we use one of the automated liquid handling platforms in the CM lab. The platform of choice depends primarily on the sample transfer volume requested and the solvent in which the molecules are dissolved.

Due to the high hygroscopicity of DMSO, platforms for the sole processing of samples in DMSO solutions are connected to a low humidity (10 % RH) air supply. To minimize the effects of evaporation, we process aqueous solutions on different platforms that are connected to standard air handling.

Regardless of the solvent, our liquid handling devices cover a broad dispensing range (2.5 nL – 1 mL) to address the needs of the researchers'

community. We opted for modular lab automation with platforms dedicated to specific working ranges.

"Low volume" transfers (nanoliter to microliter range) are managed through acoustic dispensing devices (Access SRS and Access DRS, Beckman Coulter Life Sciences, Brea, CA). "High volume" transfers (microliter to milliliter range) are processed with air-displacement pipetting devices (Fluent and Freedom EVO, Tecan, Switzerland; Biomek i7, Beckman Coulter Life Sciences). All liquid handlers have multiple dispensing settings (acoustic dispensing calibrations, liquid classes) to enable accurate and precise liquid transfers.

The modular approach is deliberate: bundling all dispensing capabilities in one single system would reduce our ability to work in parallel, and any unplanned maintenance could affect multiple workflows. With each module performing a single type of task, we retain flexibility and a higher degree of control. By using similar platforms and approaches in our laboratory automation, we provide the same workflows and deliverables (e.g., assay-ready plates) to all users independently of solvent (DMSO, water, PBS) and modality.

3.5.3. Storage temperatures

As discussed in Section 3.4.2, liquid solutions in DMSO are stored at room temperature (21 °C), above the freezing point for DMSO solutions. By staying constantly liquid, sample solutions can be accessed directly after retrieval from storage.

Aqueous solutions are stored frozen, and require thawing (at room temperature) before CM can process them. This process takes a significant amount of time (a rack holding full sample tubes takes up to 3 h to thaw) when compared to liquid handling (generally less than half an hour). To speed up our processing times, we introduced an automation-friendly rack thawing station (Heliport Plus, Box Scientific). Full racks can now thaw at room temperature in <20 min, thus benefiting all workflows that involve frozen solutions of any modality.

After thawing, we homogenize all samples before liquid handling takes place. This step is described in detail in Section 4.2, as this application made us realize the need for a homogenization step. For consistency and simplicity, we introduced this as a mandatory step in all workflows that involve samples that have been thawed.

4. Use cases

4.1. Peptides

As per small molecules, data from internal studies (unpublished) has shown DMSO to be a suitable solvent for most peptides in our company's collection. Those peptides that are not compatible with DMSO can be submitted as aqueous solutions. This allows their incorporation in existing order processing workflows and facilitates their experimental use.

In terms of order processing, peptides are treated as small molecules and—when in DMSO solution—they are stored in the same type of labware (acoustic tubes). While most of our peptides can be stored at room temperature, many need to be frozen. Acoustic tubes are not compatible with our current automated cold storage, so they are kept in a freezer (−20 °C).

4.2. Single-stranded oligonucleotides

Naked (unconjugated) or conjugated with other molecules, single-stranded oligonucleotides can be submitted as plate libraries or singletons. In all cases, the oligo content (amount of oligonucleotide vs. amount submitted) of each sample submitted is unknown.

Synthesized oligonucleotides are typically highly pure, but once lyophilized and submitted each sample contains non-oligo materials, such as solvent residues and salts. The resulting oligo content is significantly lower than the AstraZeneca business rule threshold (85 %) to include a compound in the screening collection. Utilizing these samples without further processing would lead to noncomparable experimental results. Therefore, we quantify oligonucleotide samples by measuring their optical density (Fig. 3) using a UV/VIS reader (Lunatic, Unchained Labs, Pleasanton, CA). After quantification, we normalize them to an agreed standard concentration using a bulk filler (Certus Flex, Gyger, Switzerland).

The data needed to determine concentrations (sample information, absorbance raw data, molar extinction coefficients) is stored in separate places and in different formats. We developed IT tools to calculate and retrieve molar extinction coefficients for each sample processed. We pair this information with the raw data from the UV/VIS reader and the sample-related information from LIMS. A macro-driven spreadsheet (Excel, Microsoft, Redmond, WA) brings it all together, allowing CM scientists to perform all calculations reproducibly.

The process is now well-established in CM, but we are working to improve data handling and sample processing. While functional, the current Excel tool is not intuitive and increases the complexity of CM daily tasks. We are creating a single tool to gather and process all information, simplifying the process greatly and reducing the risk of human error.

As for sample handling, iterative improvements have been part of our journey since the beginning. Our first challenge was to understand the freeze/thaw behavior of aqueous solutions. When thawed, most oligonucleotide sample solutions proved to be heterogeneous, with lower concentrations at the top of the liquid and higher at the bottom. Given that liquid handlers can be set up to aspirate samples from the surface of a liquid or the bottom, using different liquid handling methods can lead to inconsistent experimental results (Fig. 4).

To avoid such inconsistencies, we introduced compulsory homogenization after thaw. This step is performed by manual agitation and centrifugation before any sample transfers occur. While effective, this step is prone to human error (the operator might not remember to perform it), and we are considering automated alternatives.

For instance, we set off to understand whether freezing oligonucleotide solutions—a procedure dictated by the available automated sample storage in our laboratory—was mandatory.

We conducted a stability study to monitor the integrity of 81 naked antisense oligonucleotides (ASOs) when stored refrigerated (Fig. 5). We observed no significant decrease in UVxMS [11,12] purity, confirming that chilled storage (4 °C) is acceptable for at least up to two months. We consider this sufficient to cover the period during which the design-make-test-analyze (DMTA) experimental cycle [13] is conducted.

We also proved that refrigerated solutions stay homogeneous and require no additional shaking before they are used (Fig. 6).

Both datasets show that storing aqueous oligonucleotide solutions as refrigerated would be suitable from a stability perspective. This would remove the need for repeated freeze/thaw cycles and make samples available straight out of storage. A suitable automated store to support this approach is under consideration.

4.3. Double-stranded oligonucleotides

Double-stranded oligonucleotides, such as small interfering RNA (siRNA) molecules, are not compatible with the same quantification methods used for single-stranded oligonucleotides. To bypass this limitation, we established an alternative process that starts with quantification and normalization of the individual single-stranded components (guide RNA and passenger RNA, as described in Fig. 3). We then pool and anneal complementary strands, forming the desired double-stranded molecules ("duplexes," as described in Fig. 7).

To reflect the duplex formation, the sample identifiers of the two single-stranded constituents of each pool are replaced by a unique sample identifier for the newly formed molecule. The assignment of pre-registered identifiers to each duplex is done via manual IT tools. Development is ongoing to automate this data processing step.

To shorten the process time, we also invested in new equipment

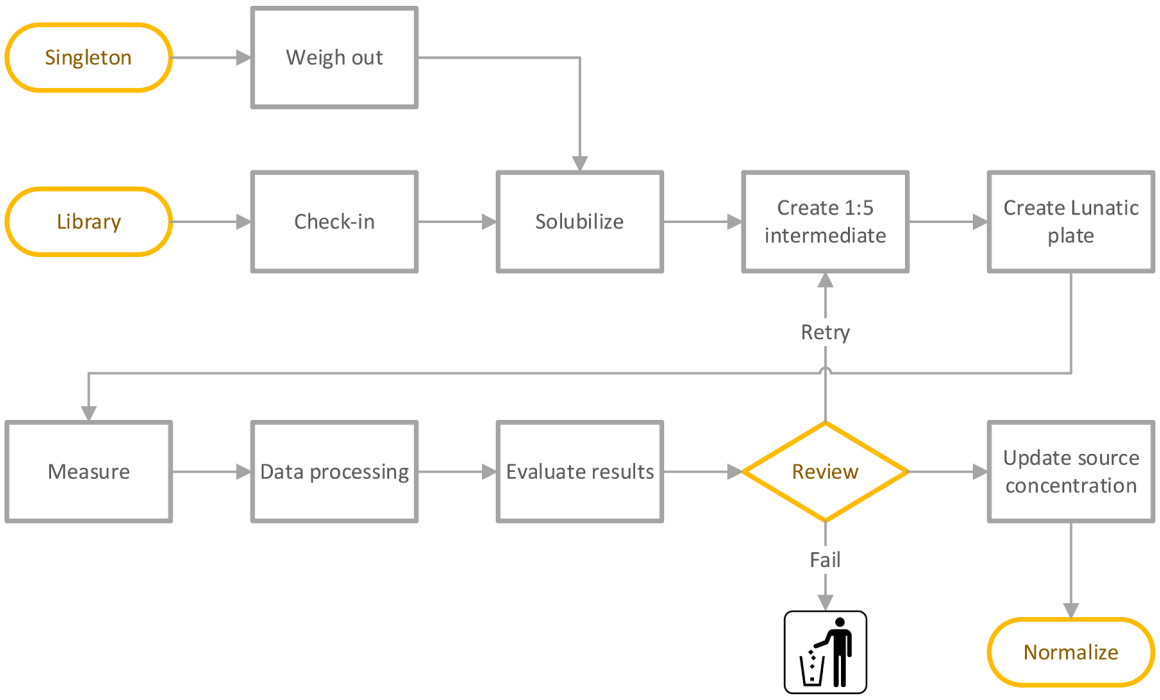

Fig. 3. Sample preparation workflow overview for single-stranded oligonucleotides, such as antisense oligonucleotides (ASO) and single-stranded RNA (ribonucleic acid). Samples are dissolved in the appropriate solvent to a nominal concentration (e.g., 3 mM) greater than the final desired concentration (e.g., 1 mM). After an additional 1:5 dilution (required to ensure compatibility with the reader's range), an aliquot of the solution obtained is transferred to a special plate for UV/VIS concentration measurement (260 nm). Once the concentration of each sample is known, samples are normalized to the final concentration.

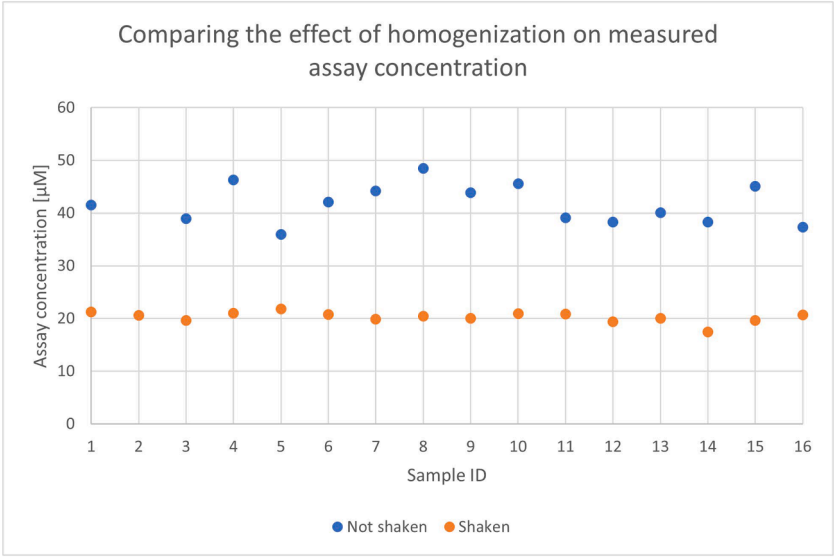

Fig. 4. Effect of post-thaw shaking. In this experiment, two copies were taken from a sample library (16 samples): a "non-shaken" copy (source tubes were thawed, then centrifuged before their contents were transferred to assay plate) and a "shaken" copy (source tubes were thawed, shaken by hand, then centrifuged before their contents were transferred to an assay plate). The introduction of a manual shaking step following the thawing greatly reduced the variability of the results and brought the assay concentrations to the approximate desired value (20 µM).

(ThermoMixer C, Eppendorf, Germany) to internalize the duplex formation process, previously performed by a different team.

5. Conclusions

The introduction of aqueous workflows within Compound Management has had a transformative impact on AstraZeneca's early pharmaceutical research program. Through strategic investments and leadership support, we can now manage a much broader variety of modalities and support novel science for the ultimate benefit of patients worldwide.

However, our journey has only just begun. The methods and equipment we deployed are mere tools to support a rapidly evolving scientific landscape. In fact, other modalities not discussed herein have already appeared on the horizon and might find their way to CM soon.

Mindful of the fast pace of scientific research, we attempted to future-proof our equipment by adopting a modular approach to lab automation. As innovative technologies and requirements emerge, we

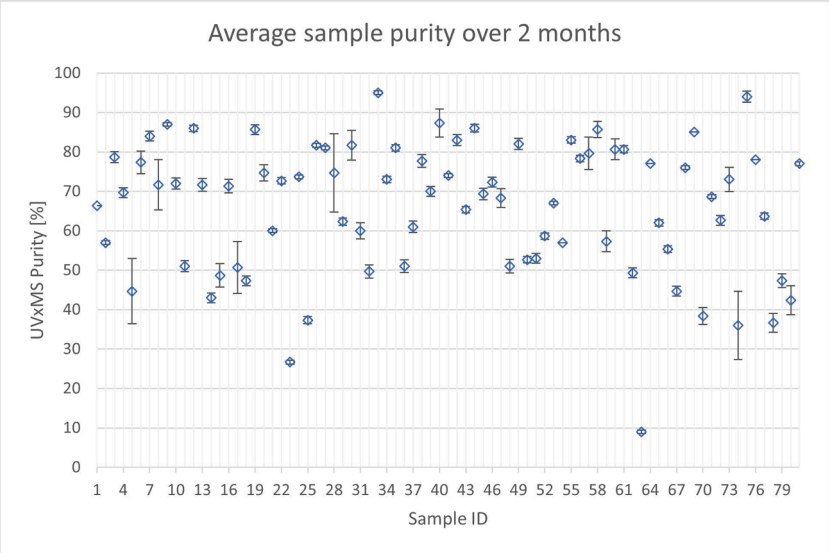

Fig. 5. UVxMS (UV peak area multiplied by spectral purity) purity analysis data of 81 single-stranded oligonucleotide sample solutions over the observation period of 2 months. We measured purity of each sample when it was first dissolved and at other two occasions one month apart. The samples have been kept chilled (in a refrigerator at 4 °C) throughout the study. The graph shows the averaged purity values for each of the samples in the study and their error bars, demonstrating the stability of the samples during this period. Only a few samples showed relatively larger variability. This can be explained as experimental errors at the time of analysis.

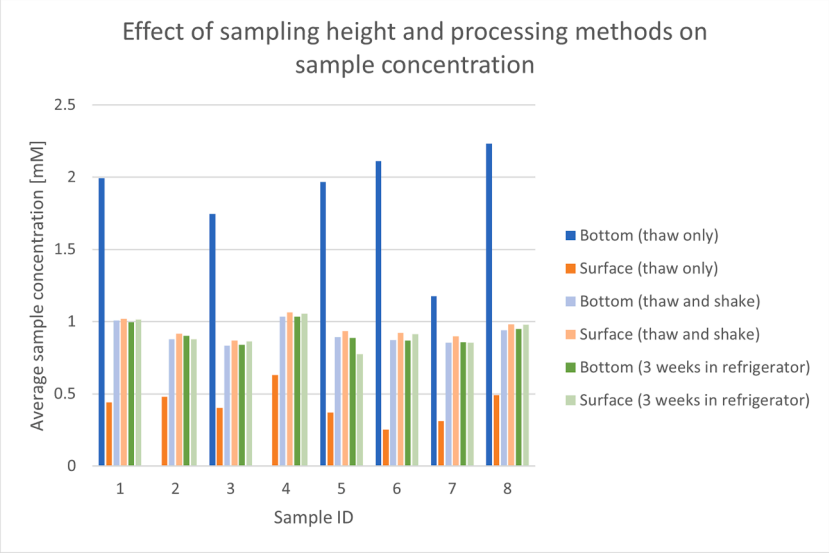

Fig. 6. Average oligonucleotide concentrations measured at different sampling heights within the storage tube (bottom vs. top of the liquid) and using different processing methods. Simply thawing a frozen sample generates internal gradients in the sample tubes, leading to higher concentrations at the bottom of the liquid and lower at the surface. As per current practice, post-thaw shaking effectively homogenizes the contents of each sample tube. When a sample is chilled, its properties remain unchanged over the observation period (3 weeks). No gradient formation could be detected, and all chilled samples show the same behavior as the current thawing and shaking method.

will be able to introduce or replace equipment without major laboratory and building redesign. This will help to contain costs and promote sustainability.

We also worked to introduce similar flexibility in the informatics that support aqueous workflows by introducing a variety of generic therapeutic modality descriptors that are carried throughout the different IT systems, including ones before and after the CM process.

Being pioneers comes with challenges beyond the scientific and technological aspects described so far. Starting in a new domain can bring about frustrating feelings, like suddenly not being experts anymore or wishing for streamlined operations from day one.

It is easy to forget that developing knowledge and understanding requires time, patience, and cooperation. It is therefore crucial to leverage and support relevant knowledge-sharing platforms, journals, and communities to foster collective scientific advancements. This is where we can discuss the effective management of new modalities compound collections and agree upon new best practices to move science forward together.

CRediT authorship contribution statement

Silvio Di Castro: Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Conceptualization. **Martin L. Svensson:** Writing – review & editing, Methodology, Data curation.

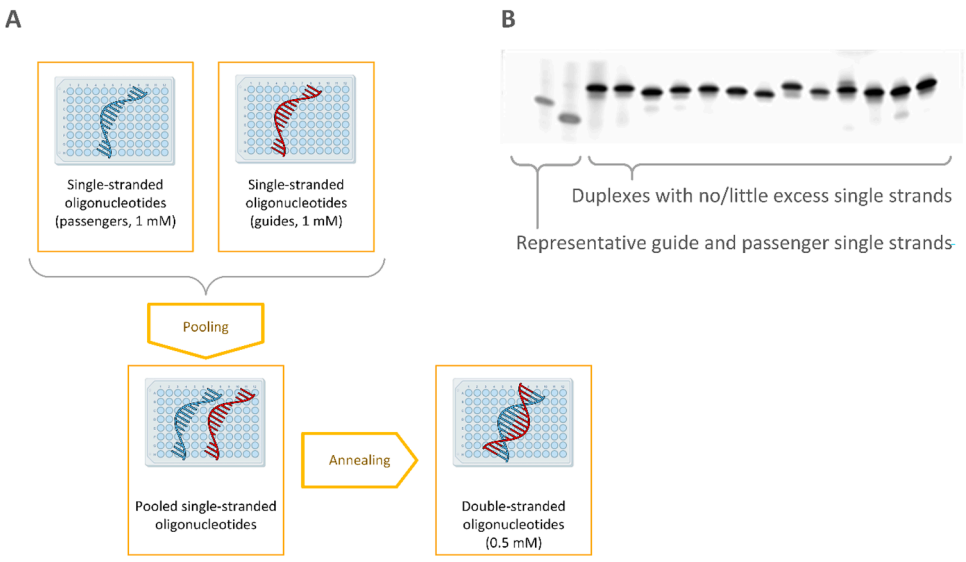

Fig. 7. A) Duplex formation process, starting with pooling of single-stranded oligonucleotide solutions that have been quantified and normalized to a standard concentration (1 mM). After annealing, each double-stranded oligonucleotide is assigned a new identifier and becomes available for experimental use. B) Gel electrophoresis image showing the predominance of double-stranded oligonucleotide after annealing and near-full conversion. Confirmed by empirical evidence, the concentration of each duplex is therefore assumed to be half the value of the starting single-strand concentration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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