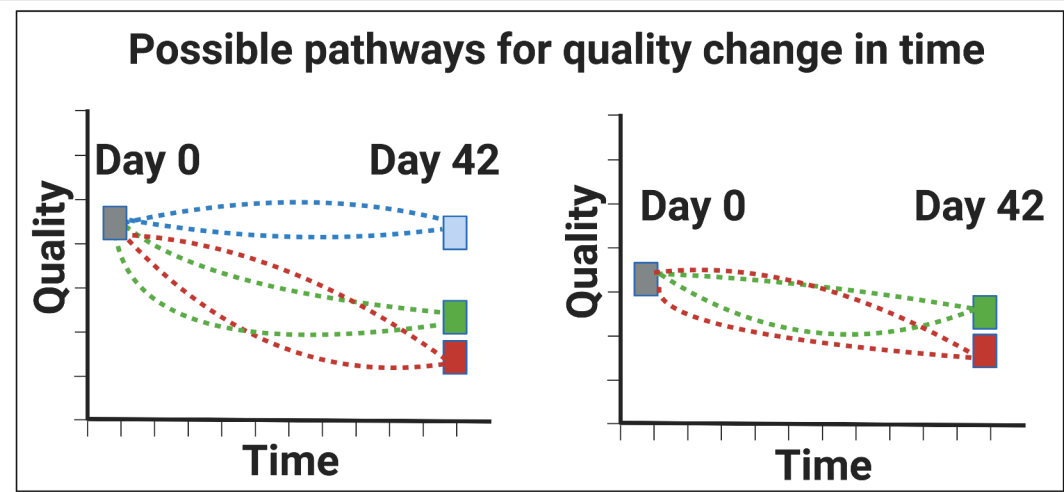
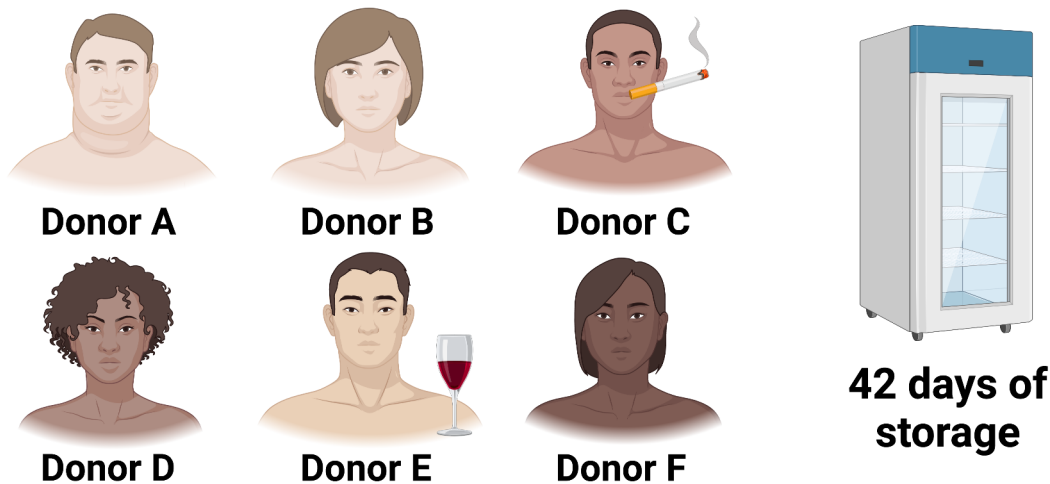


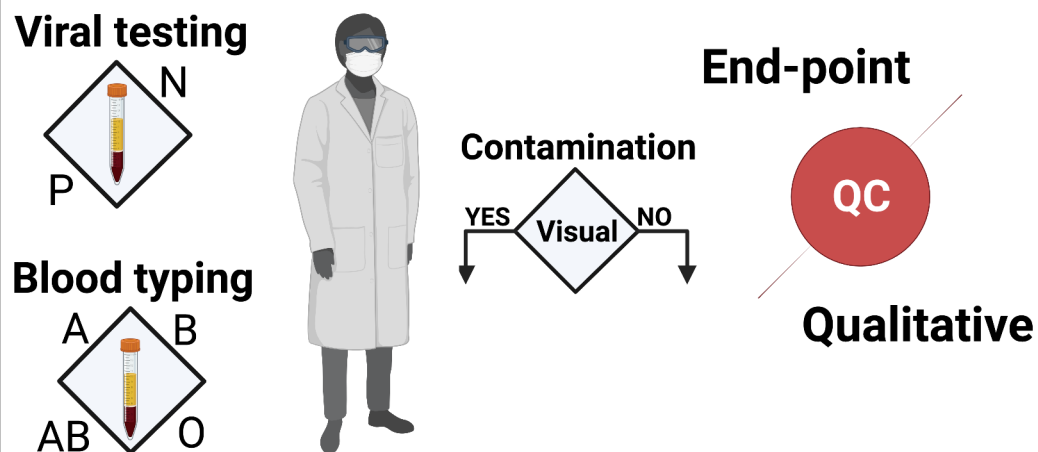
Donation and Storage



Transfusion

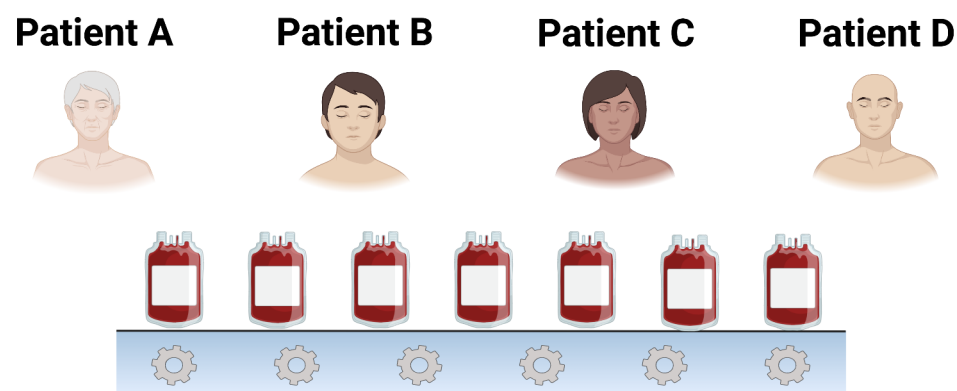
Current Workflow

Conventional Assessments



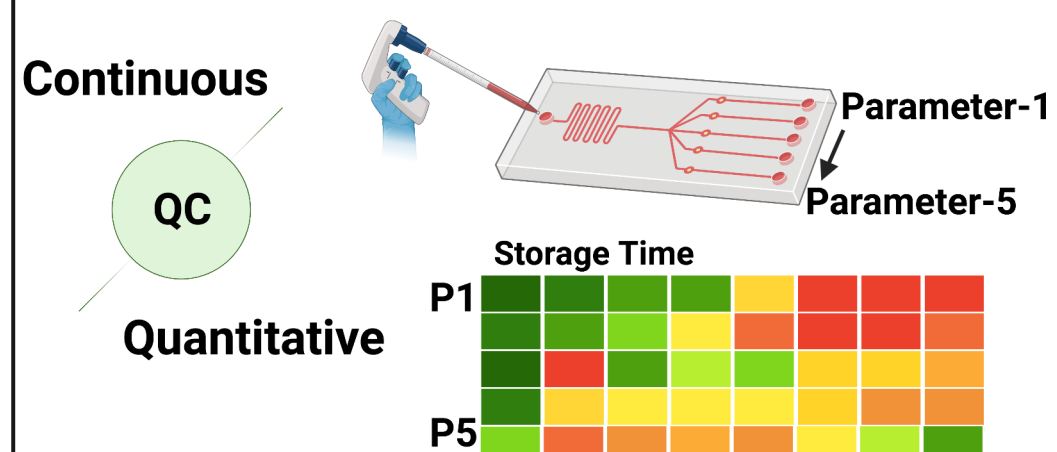
FIFO/LIFO Allocation

First-In-First-Out (FIFO) or Last-in-First-Out (LIFO)



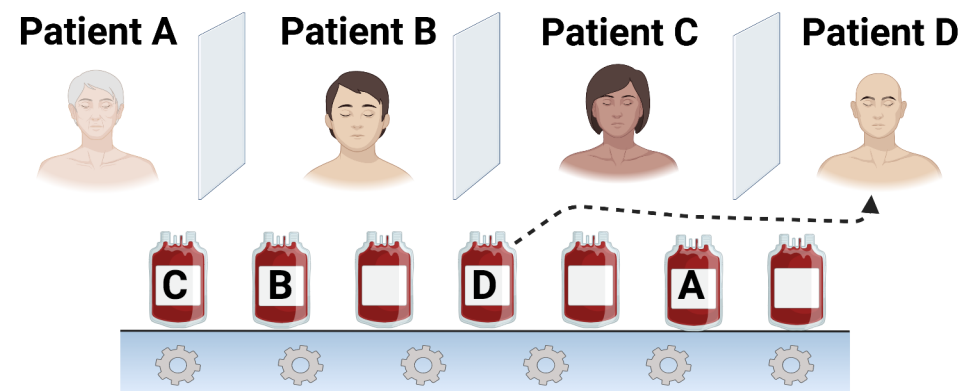
Future Workflow

Novel Assessments



Data-driven Allocation

Matching "RBC Unit" Properties to "Patient" Needs



Biochemical



ATP

2,3 DPG

Nitric Oxide

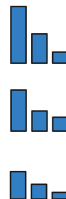
Donor A



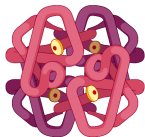
Donor B



Donor C



Oxidative



MDA

ROS

Glutathione



Morphological



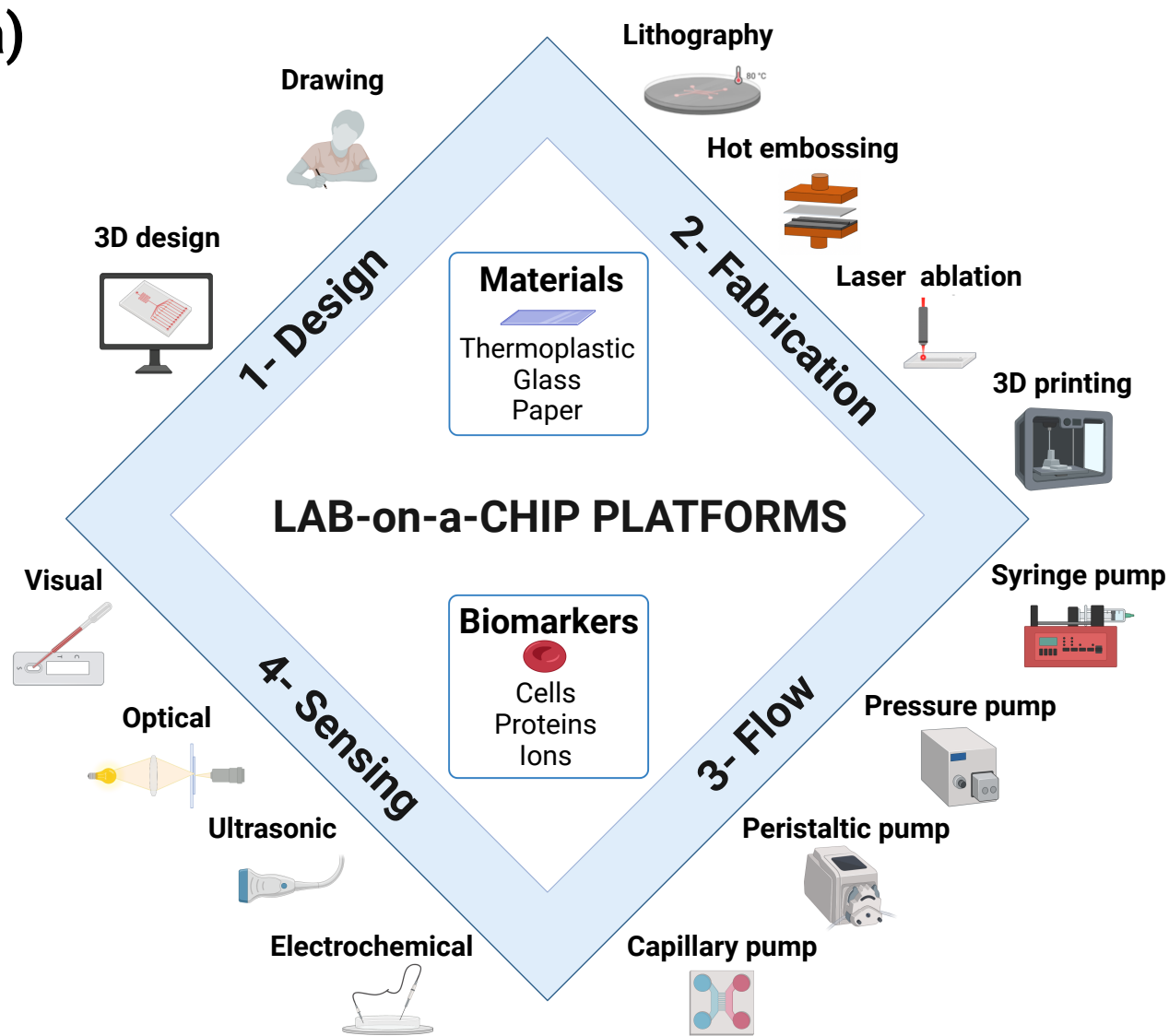
Hemolysis

Deformability

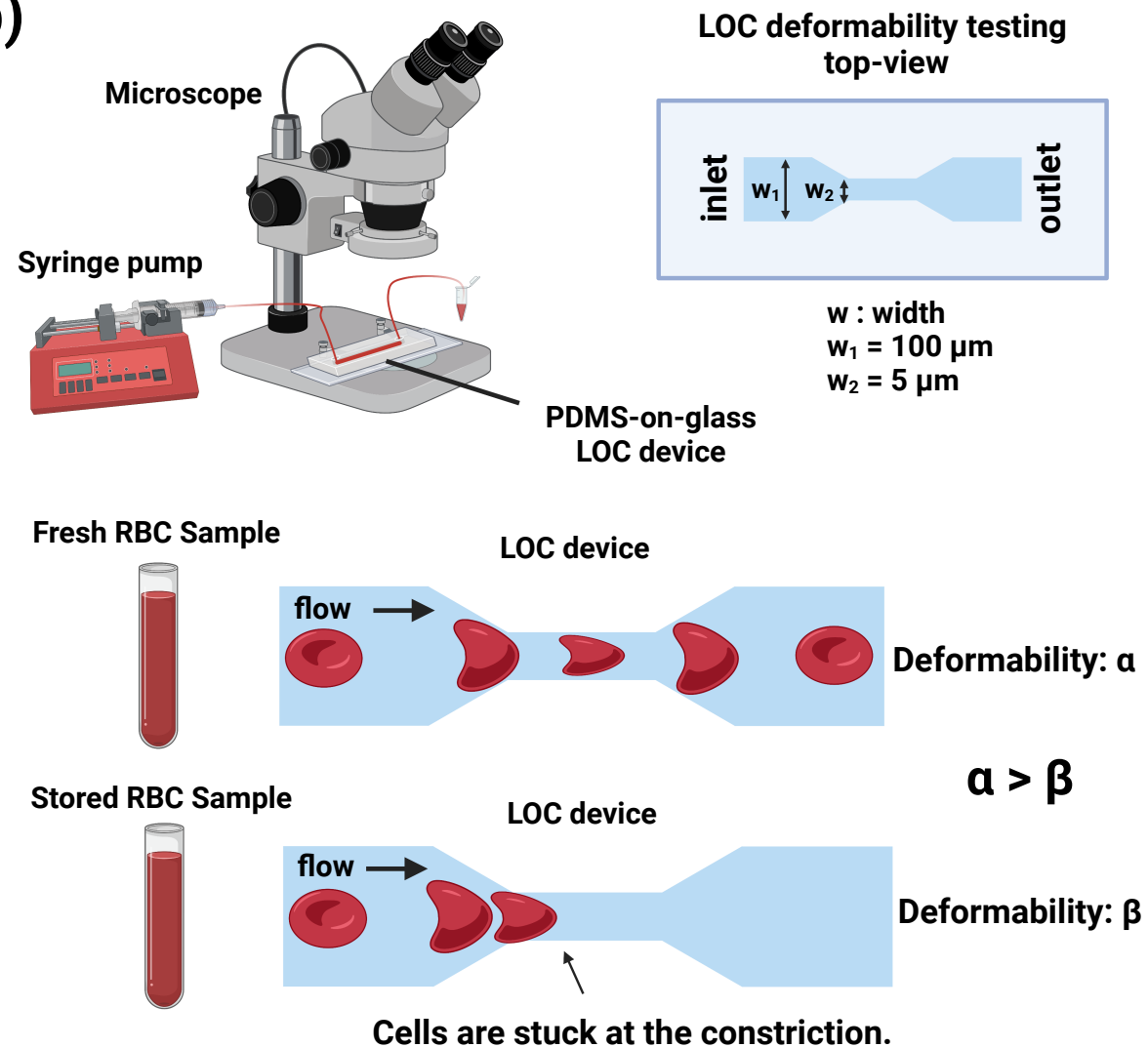
Microvesicles



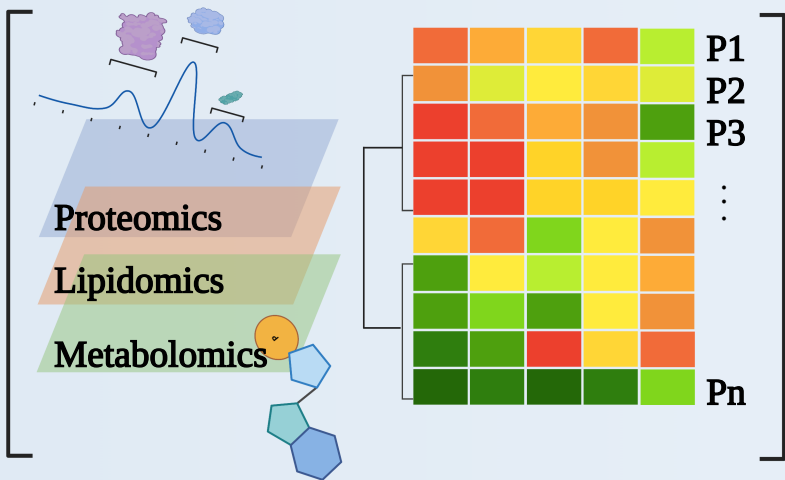
a)



b)

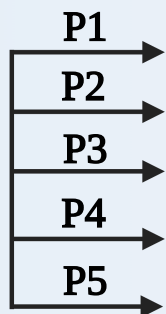


OMICs

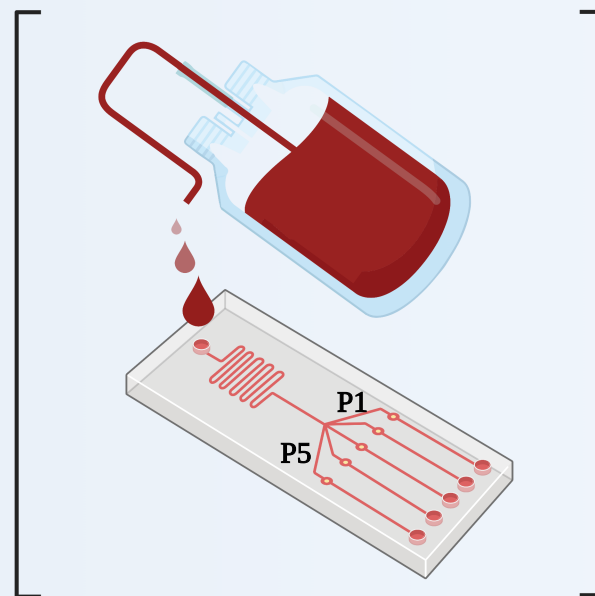


Machine Learning

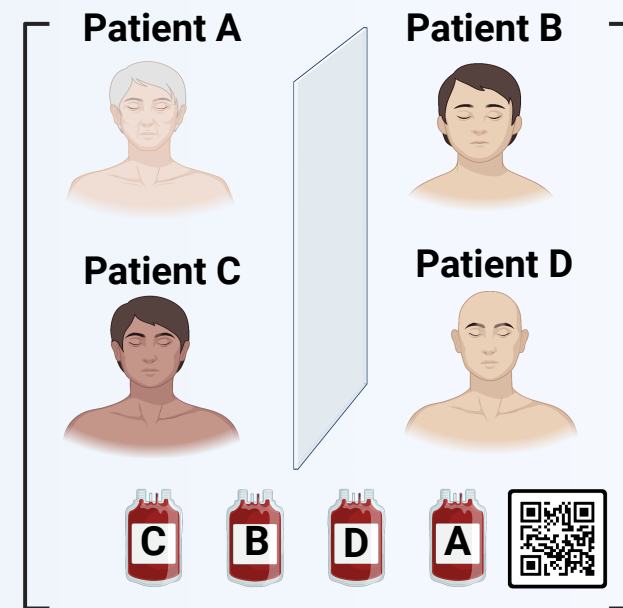
Quality Indices



Integrated Lab-on-a-Chip Technologies



Personalized Transfusion



Assessment of stored red blood cells through lab-on-a-chip technologies for precision transfusion medicine

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38 Abstract

39 Transfusion of red blood cells (RBCs) is one of the most valuable and widespread treatments in modern medicine.
40 Lifesaving RBC transfusions are facilitated by the cold storage of RBC units in blood banks worldwide. Currently,
41 RBC storage and subsequent transfusion practices are performed using simplistic workflows. More specifically,
42 most blood banks follow the “first-in-first-out” principle to avoid wastage, whereas most healthcare providers prefer
43 the “last-in-first-out” approach simply favoring chronologically younger RBCs. Neither approach addresses recent
44 advances through -omics showing that stored RBC quality is highly variable depending on donor-, time-, and
45 processing-specific factors. Thus, it is time to rethink our workflows in transfusion medicine taking advantage of
46 novel technologies to perform RBC quality assessment. We imagine a future where lab-on-a-chip technologies
47 utilize novel predictive markers of RBC quality identified by -omics and machine learning to usher in a new era of
48 safer and precise transfusion medicine.

49
50 **Keywords:** red blood cell, storage lesion, biopreservation, transfusion medicine, lab-on-a-chip, metabolomics,
51 machine learning, precision medicine

52 Introduction

53 Red blood cells (RBCs) constitute more than 90% of the cellular blood content in humans and perform one of the
54 most essential roles for life, oxygen transport. RBC transfusion is a common preventive and therapeutic procedure
55 with 36 thousand units transfused daily in the US alone (1–3). Here, we review the decades-old quality control
56 practices for stored RBCs and contend that advances are warranted using novel lab-on-a-chip (LOC) technologies
57 to enable precision transfusion medicine.

58
59 Blood transfusion therapy has evolved into today's practice over the last four centuries (4). Consequently, many
60 patients and practitioners mistakenly assume that current transfusion workflows have reached their final form.
61 Historically, the first convincing demonstrations of blood transfusion and its utility occurred during the First World
62 War. These demonstrations were facilitated by advances in blood storage practices (5) including additive solutions-
63 enabled extended storage (6, 7), and later plastic blood bags (8) that permitted lighter weight and reduced
64 contamination.

65
66 The World Health Organization (WHO) reports that whole blood donation exceeds 120 million units annually, in
67 response to a massive global need (3). The logistics of the RBC supply-and-demand chain are sustained through
68 commonly agreed-upon storage practices in blood banks. The US Food and Drug Administration (FDA) and the
69 Centers for Disease Control and Prevention (CDC) are involved in keeping the blood supply safe by mandating
70 quality control and assurance parameters, and monitoring incidents during manufacturing and adverse post-
71 transfusion reactions (9). These US agencies, along with their international counterparts, have been instrumental in
72 establishing quality and product clearance regulations for RBC storage and transfusion. Specifically, the current
73 practice for RBC banking is storage at 4 °C for 42 days (10–12). This practice is based primarily on the FDA-
74 mandated requirements that RBCs undergo less than 1% hemolysis during storage and that at least 75% of
75 transfused RBCs survive at 24 hours after transfusion (13, 14). The slow pace of change and improvements to these
76 common practices, however, should not imply that RBC storage and transfusion strategies are optimal. It rather
77 signals a lack of low-cost and translatable tools to assess quantitatively, (*i.e.*, objectively via unbiased
78 measurements), the quality of RBC units.

79
80 Stored RBCs exhibit unique donor-, time-, and processing-dependent metabolic and morphological changes
81 collectively termed storage lesions (15, 16) (**Fig. 1**). For example, increased cellular acidity inhibits glycolytic
82 pathway enzymes, depleting adenosine triphosphate (ATP) reserves. A decline in 2,3-diphosphoglycerate (2,3-
83 DPG) changes oxygen kinetics (17, 18). Morphological changes and oxidative injuries jointly cause RBC
84 breakdown (*i.e.*, intravascular hemolysis) or increased sequestration in the spleen (*i.e.*, extravascular hemolysis)

(19, 20). Overall, these lesions imply reduced viability and impaired functionality of stored RBCs, negatively impacting their efficacy and safety in transfusion.

Several studies in the early 2000s have highlighted risks associated with the use of older stored RBCs versus fresh units (21–26). Others have challenged such claims using large-scale randomized clinical trials (27–31). However, these trials have also drawn criticism due to their study design and intrinsic limitations (poor delineation of storage time, mortality as the primary outcome, *etc.*) (27, 28, 32). Thus, a consensus on the clinical impact of the time-dependent RBC storage lesion has not yet been reached. This stems from a lack of data on the actual quality (real age (33)) of RBC units beyond their storage time (chronological age) (**Fig. 1**). Following initial pathogen screening and blood typing, only some arbitrary RBC units – if any – go through destructive (sacrificing the tested RBC units) quality control assays, and only visual inspections are conducted as end-point quality control release tests (**Fig. 1**). However, recent studies indicate that the temporal evolution of storage lesions is dependent on donor variability factors such as sex, age, ethnicity, genetic disposition, body mass index, metabolism, and lifestyle (34–38). The severity of adverse effects from RBC transfusion also depends on recipient factors (*e.g.*, health state, transfusion volume and frequency). Objective assessment of storage lesions can lead to RBC quality and “real age” determination and rational decision-making for donor-to-recipient selection. Doing so will improve transfusion safety and efficacy, and potentially lead to better storage practices.

Two emerging approaches have been used to quantitatively assess the RBC storage lesions: -omics and machine learning. Specifically, -omics methods (proteomics, lipidomics, metabolomics) that provide comprehensive characterization and quantification of biomolecules via mass spectrometry have been used to study RBC changes upon storage. These studies have led to identification of potential biomarkers to assess RBC storage injuries (39). Similarly, machine learning combined with imaging flow cytometry has captured the progression of storage-induced changes in RBC morphology and deformability (40, 41). Together, -omics and machine learning technologies have the potential to identify key quality parameters that are predictive of clinical transfusion success for stored RBCs. Yet, the adoption of -omics on a large scale for routine use is hindered by their cost and analysis time, though these hurdles are overcome with the introduction of high-throughput -omics.

Lab-on-a-chip (LOC) technologies offer an alternative for routinely measuring RBC unit quality. These LOC technologies allow miniaturization of analytical assays with the benefits of sensitivity, specificity, ease of use, portability, rapid turnaround, and high-throughput (42–45). As such, LOCs can become a new frontier for assessing stored RBC products using quality parameters that result from combined -omics and machine learning studies. More specifically, LOCs can measure and immediately report these key quality parameters for every stored RBC unit to enable safer transfusion (without any unintended adverse consequences) by matching the properties of the RBC unit to the needs of the patient (right unit to right patient). Nevertheless, despite their increasing adoption and

commercial successes in other areas (pregnancy, glucose, and rapid COVID-19 tests), LOCs are scarcely used to assess stored RBCs. Yet, recent research is demonstrating the potential of LOCs to improve RBC safety assessment (46–53).

This Perspective introduces the unexplored intersection of i) RBC storage lesions, ii) the need for RBC quality assessment, and iii) the potential for integrated LOC technologies to provide for safer transfusion therapy. First, we highlight the issues regarding the quality of stored RBCs. Specifically, we and others posit that stored RBCs, under current practices, are not always safe to transfuse due to donor, time, and processing factors. This is especially problematic for critically ill patients, those on chronic transfusion regimens, and surgeries where large volumes of RBCs are transfused. The lack of assessment of stored RBCs before transfusion on a unit-by-unit basis represents a failure, especially in this era of precision medicine. We then discuss solutions to this critical issue using accessible and robust LOC platforms and their convergence with other state-of-the-art approaches (-omics and machine learning).

Current Practices in Red Blood Cell Storage and Quality Assessment

Storage of Red Blood Cells

RBCs undergo donor-, time, and processing-dependent metabolic, biochemical, and morphological injuries during their extended storage, collectively termed storage lesions (54–56). These include progressive increases in hemolysis (57); intra- and extra-cellular acidification (58); depletion of glucose (15); accumulation of extracellular potassium and intracellular calcium ions (54); alteration in osmotic fragility; depletion of intracellular energy and redox pools (reduced and oxidized glutathione) (55); and decrease in deformability (59) (**Fig. 2**). The identification of storage lesions and efforts to mitigate them have led to the development of improved storage solutions to extend storage time for banking purposes. As a result, the current widely used storage method for RBCs is cold storage at 4 °C for up to 42 days of storage in various additive solutions (SAG-M, AS-1, AS-3, *etc.*) (54).

Quality Assessment of Stored RBCs

There are only two criteria mandated by the FDA and international counterparts for the quality and acceptable storage of RBCs: i) at least 75% of autologous RBCs must survive at 24 hours *in vivo* after transfusion, and ii) there must be less than 1% hemolysis in the storage container (13, 14). Novel RBC products – featuring new storage solutions, methods, or other improvements – must meet these FDA criteria for clinical use. The standard practice of up to 42 days of cold storage for RBCs is primarily driven by the need to meet these two criteria. In 2008, several clinical retrospective studies presented data that suggested chronologically old RBCs (stored for more than two weeks) were associated with worse outcomes upon transfusion in different patient groups. In contrast, subsequent randomized clinical trials indicated that there are no significant differences between transfusing chronologically

fresh versus older stored RBCs. In the following paragraphs, we summarize the findings of these different studies and the ongoing discussion on stored RBC quality and their chronological age. Nevertheless, the different views on this question stem from a lack of data on the real age – *i.e.*, quantitative and objective metrics of quality – of RBC units beyond their chronological age. In this Perspective, our goal is to urge that the field adopt robust and routine quality assessment of stored RBCs.

According to the 2008 retrospective clinical study, transfusion of RBCs cold-stored for more than two weeks was associated with increased risks of postoperative complications and reduced survival after cardiac surgery (21). A similar result was found for trauma patients and corroborated by further studies (22, 23). Specifically, trauma patients administered three or more RBC units within 24 hours of their hospitalization had a higher mortality risk when relatively older (>14 days) RBCs were used. A follow-up study indicated inhibition of regional microvascular perfusion in anemic (but otherwise stable) patients when older (>21 days) RBCs were used (24). However, these studies were retrospective and hence lacked proper randomization. Nevertheless, their findings prompted agencies such as the US National Heart, Lung, and Blood Institute (NHLBI) to reexamine the suitability of the current practices (25, 60).

Subsequent randomized clinical trials (RCTs) on a subset of patient groups indicated no inferiority of selective transfusion of freshest available RBC products vs standard of care for various patient groups (27–31). These studies contrast with the previous retrospective and observational clinical studies and with recent animal studies that predict high mortality in disease models of animals when older (42-days-old) RBCs are transfused (61, 62). While RCTs are regarded as the gold standard for clinical studies, several issues were raised regarding these RCTs (27, 28). These RCTs were conducted in specific patient groups, and their results may not be generalizable to all patient populations. Also, none of the RCTs evaluated the effect of transfusing RBCs stored between 35-42 days, which is the period other clinical trials have questioned (63). In fact, Pereira *et al.* suggested that the RCTs lack enough power to resolve the issue of the “old” RBCs (32) and recommended reducing the maximum storage time to 35 days.

The quality of stored RBCs may not necessarily be dictated by their chronological age. As we already indicated, the RBC quality varies based on not only storage time but also donor, processing, storage solution, and environmental factors. Indeed, within individual RBC units, there are heterogenous subpopulations with unique structural, functional, and metabolic dissimilarities due to donor-dependent differences in hematopoiesis and *in vivo* eryptosis (64). Similarly, the needs of each patient for transfusion vary depending on several factors, including their own disposition, condition, and the anticipated operation. Therefore, the focus when using stored RBCs should be on assessing the quality and thus the transfusibility of each stored unit for the particular patient in need, whether the storage lesions be due to donor, time, or processing factors. These considerations have fueled the debate on

188 personalized transfusion medicine approaches, a key goal in the agenda of the NHLBI, as discussed in the State of
189 the Science in Transfusion Medicine Workshop (60). Thus, rather than an overly simplistic “first-in-first-out” or
190 “last-in-first-out” approach, a data-driven selection of stored RBC units for each patient will prove superior (**Fig.**
191 **1**). The routine use of such a data-driven approach will reduce transfusion complications, improve outcomes, and
192 enable better allocation of RBC units for every patient.

193 State-of-the-Art Assessment Approaches via -Omics and Machine Learning

194 -Omics for Transfusion Medicine

195 There has been progress in recent years in our understanding of storage lesions using -omics technologies (55) –
196 especially metabolomics (65). This has been primarily driven by advancements in high-throughput mass
197 spectrometry (66), a technology allowing the analysis of large populations. A paradigmatic example is the Recipient
198 Epidemiology and Donor Evaluation Study III – RBC Omics (REDS). This study enrolled ~14 thousand healthy
199 donors across four US blood centers, and performed both donor genomics and metabolomic analyses of stored RBC
200 units (67). These data were gathered in parallel to laboratory measurements of hemolysis (35, 68), clinical
201 measurements of iron metabolism (69), and recipient databases for functional read-outs (*e.g.*, post-transfusion
202 hemoglobin increments (70)), ultimately resulting in what is defined as a “vein-to-vein” database (71).

203
204 The opportunity to generate a wealth of data through metabolomics testing of thousands of RBC units in the REDS
205 study has allowed the field to appreciate the impact of donor biological variables (sex (34), age (35), ethnicity (72),
206 body mass index (36), testosterone levels (37)) on RBC storage quality. Interestingly, the genetic background of
207 the donor significantly impacts the heterogeneity observed in storage profiles. Such is the case for common
208 polymorphisms – *e.g.*, glucose 6-phosphate dehydrogenase deficiency that affects ~7% of the world population
209 (73). Similar considerations apply to other common polymorphisms, such as those found in beta-thalassemia minor
210 (74), band 3 (75), and glutathione peroxidase 4 (76). Metabolomics has also demonstrated the impact of processing
211 (*e.g.*, leukoreduction/leukodepletion (77)), and the blood donor “exposome” on the onset, progression, and ultimate
212 storage lesion severity (78). Specifically, based on -omics studies, we now appreciate the impact of factors such as
213 donor exposure to smoking (38), alcohol (79), and caffeine (80) on RBC storage quality. Furthermore, -omics has
214 shown that certain drugs – that are not grounds for donor deferral – are routinely identifiable in stored blood
215 products, with the potential to negatively impact storage quality and promote untoward consequences in transfusion
216 recipients (78).

217
218 Overall, large-scale -omics studies have paved the way for personalized medicine, revealing that storage duration
219 (chronological age) might be less relevant than the “real age” (metabolic age) of the unit (81). Applications of -
220 omics to transfusion medicine have recently yielded potential new metrics of the storage lesion and post-transfusion

performances. These include post-transfusion recovery (82), storage or oxidative hemolysis (34, 73), or oxygen kinetics (17). These parameters can now drive the development of cost-effective, targeted strategies to monitor theoretically all the >100 million blood units donated yearly worldwide.

224

Machine Learning for Transfusion Medicine

Advanced technologies to assess RBC quality and state – such as -omics – generate complex multivariate data (83). This data-intensive (*i.e.*, “big data”) approach to assessing stored RBC quality necessitates using computational statistical techniques to objectively extract meaningful patterns from these data sets (84). Such combination of big data and computational statistical tools is critical to biological interpretation and aiding the data-driven selection of stored RBCs for precision transfusion medicine (85).

231

Among computational approaches, machine learning (ML) has shown promise in accurately classifying complex multivariate data and aiding critical decision-making in biomedicine (86–88). Of interest to transfusion medicine, ML was recently leveraged to predict preoperative RBC demand and the objective assessment of stored RBC quality (40, 89). One study involved a retrospective analysis of ~130 thousand surgery patients using seven ML algorithms (81). This led to a more objective approach for predicting the quantity of RBCs needed by each patient, compared to clinicians making the same decisions without ML. In another study, brightfield images were used to distinguish between RBC morphologies and predict stored RBC quality using ML algorithms (40). ML results correlated better with physiological tests of RBC quality, compared to subjective expert annotation. Further examples at the intersection of transfusion medicine, big data, and ML include the improved quality monitoring of stored RBC units via the identification of new injury markers (40) and the development of new products (90); novel storage additives (91) and strategies (92); rejuvenated units (93).

243

These advanced technologies, especially -omics, have enriched our understanding of storage lesions and led to the development of potential new metrics for RBC quality assessment. Currently, these technologies are limited to research, predominantly due to the relatively low accessibility of the instruments and the complexity of the data. Although the ML toolbox has demonstrated success in tackling such data, implementation of ML-based solutions in healthcare will require expertise and installation of software infrastructure. This necessitates an alternative approach that can harness the results of -omics and ML and provide quick, non-invasive, economically feasible, and user-friendly quality measurements on stored RBC units.

Lab-on-a-Chip Technologies for the Assessment of Stored Red Blood Cells

Overview of Lab-on-a-Chip Technologies

Lab-on-a-chip (LOC) platforms are miniaturized fluidic systems that facilitate chemical and biological analyses with sample volume requirements as low as pico-to-nanoliters (**Fig. 3a**) (42–45). Many materials (polycarbonate, paper, polydimethylsiloxane) are available for applications with different requirements. Fabrication methods are dictated by the material choice and include lithography, hot embossing, and 3D printing. Chemical and biological analyses require the manipulation of fluids facilitated by components including i) pumps to drive, ii) valves to selectively direct, and iii) mixers to combine fluids (94, 95). Optical, ultrasonic, electrochemical, electrical, and other analytical components can also be integrated (96–102), further expanding the LOC toolbox for sensing biomarkers (proteins, metabolites, *etc.*).

During recent decades, LOCs have enabled several transformative technologies that have improved public health and become commercial successes (45). These range from nucleic acid sequencing (103) to rapid diagnostics (103), biochemical analyses (104), and antibody selection (105). Today, LOCs are also indispensable in single-cell -omics (106), synthetic biology (107), liquid biopsy (108), organs-on-chips (109), and wearable sensors (110). Despite their increasing utility, LOCs have not yet made a translational impact in transfusion medicine. However, the promising examples described below demonstrate the utility of LOCs to quantify stored RBC quality.

LOC Platforms for RBC Quality Assessment

Microfluidic platforms have been utilized to characterize storage-induced alterations in RBCs, with a predominant focus on the stiffness analysis of stored RBCs (**Fig. 3b**). The simplest approach allows RBCs to adhere to glass in a microchannel (46). A pressure-driven flow is then used to deform the adherent RBCs and measure their shear modulus via image analysis. Similar shear modulus measurements are also possible by deforming RBCs flowing through a constriction and quantifying their subsequent recovery in a wider channel through image analysis (47, 48). These suspension-based approaches enable a higher throughput than the adherent cell approach. Together, these studies have confirmed storage-induced increases in RBC stiffness.

An alternative approach to direct measurement of shear modulus is the margination and subsequent fractionation of RBCs of different deformability. Several groups have developed microfluidic cell sorters where deformable RBCs migrate toward the channel center, and stiffened ones toward the sidewalls in laminar flow (49, 50). These studies show significant correlations between storage-induced osmotic fragility and storage-induced stiffness in RBCs (49), and deduce storage time from the fraction of stiff RBCs (50).

284 Such deformability-based fractionation of stored RBCs can also be achieved with higher fidelity using a
285 microfluidic ratchet device (51). This device features an array of constrictions (tapered micropillars) and 12 outlets.
286 The size of constrictions varies gradually perpendicular to the sample flow direction. Using an oscillatory crossflow,
287 cells can be filtered through constrictions until they reach a limiting constriction size. The device can fractionate
288 cells into 12 stiffness levels to study donor and storage-induced variability in RBC stiffness. A micropillar array of
289 reducing constriction size along the flow direction can also be used to measure RBC deformation (52). This device
290 (OcclusionChip) mimics a transition from capillaries to microcapillaries and reports the number of occluded
291 constrictions in each array zone. This allows the quantification of deformability associated with microcapillary
292 occlusion (53). This device has been integrated with microelectrode arrays to enable real-time, electronic, portable
293 read-out of RBC microcapillary occlusion via the Microfluidic Impedance Red Cell Assay (MIRCA) (111). The
294 American Medical Association (AMA) has issued the first and only CPT® Proprietary Laboratory Analyses (PLA)
295 Codes (CPT® 0303U and 0304U) for measuring RBC health using this LOC technology.

296

297 An active research area using LOCs is measuring RBC metabolite release. Two examples feature measurements of
298 the ability of stored RBCs to i) release oxygen (112) and ii) release ATP and nitric oxide (NO) in circulation rather
299 than measuring the intracellular levels (113, 114). Measurement of oxygen release rate is possible using precisely
300 controlled microenvironments at the single-cell level in LOCs via luminescent probes (112). Similarly, ATP and
301 NO can be facilitated by the chemiluminescent detection of the luciferin/luciferase reaction for ATP and
302 amperometric (113) or fluorogenic detection for NO (114). Indeed, LOCs can measure ATP and NO release from
303 RBCs (113) and quantify the downstream effects of ATP release – stimulation of NO release by endothelial cells –
304 by integrated microfluidic components (114). These studies, together, have assessed the quality of stored RBCs
305 with different glucose levels and compared normoxic and hypoxic conditions for storage.

306

307 An inevitable and detrimental effect of RBC storage is oxidative stress, which usually correlates well with hemolysis
308 and, thus, the “real age” of stored RBCs. A study shows that oxidative stresses of stored RBCs can be predicted
309 from the dielectrophoretic mobility of the cells. Specifically, an optical trap was used to immobilize RBCs in a
310 microfluidic chip, and then a dielectrophoretic trap was activated to measure the dielectrophoretic force on each
311 RBC (115). This study indicates that longer-stored RBCs and RBCs subjected to oxidative stress have significantly
312 lower dielectrophoretic mobilities compared to fresh and shorter-stored RBCs.

313 A Convergent Future of RBC Quality Assessment for Precision Transfusion 314 Medicine

315 How best to assess the freshness, quality, and transfusion efficacy of unique RBC units is still unresolved. The
316 current RBC transfusion workflow fails to assess the donor-, time-, and processing-dependent quality (“real age”)

of RBCs prior to transfusion. The inability to use real age dismisses variables that affect RBC quality and the suitability of the unit for the particular patient. Consequently, a long list of complications, ranging from sepsis and multi-organ failure to other morbidities, and ultimately mortality can occur following RBC transfusion. This is especially true for critically and chronically ill patients who require multiple transfusions.

We have highlighted the lack of quantitative assessment of stored RBCs before transfusion on a unit-by-unit basis, a notable failure in this era of personalized medicine. We foresee that, over the next decade, state-of-the-art technologies such as -omics and machine learning can be combined to first identify a set of key quantitative quality metrics (*i.e.*, a quality index) for stored RBCs. This work is already underway, led by the pioneers in the field (116). Subsequent translation of such quality metrics to a non-invasive and continuous assessment of RBC quality during storage could enable better and more appropriate allocation, less waste, and, most importantly, safer transfusion with better efficacy for all patients.

Lab-on-a-chip platforms (LOCs) are now mature enough to be integrated with advanced biosensors featuring flexible electronics and wireless transmitters. We anticipate that such integrated LOCs can be installed on every RBC storage bag and enable measurements of multiple biochemical and morphological parameters. Accordingly, we envision a future where LOCs leverage quantitative quality indices – identified by -omics and machine learning– to monitor stored RBC quality continuously and affordably (**Fig. 4**).

Limitations and Challenges for Precision Transfusion Medicine

The path to our envisioned convergent future (**Fig. 4**), where integrated LOCs on each RBC unit provide quality assessment prior to transfusion, is not without its hurdles and challenges. The ASSURED criteria for LOC platforms stand for “affordable, sensitive, specific, user-friendly, rapid and robust, equipment free, and deliverable to end-user” (117). As summarized in this Perspective, the current LOC platforms developed for stored RBC assessment present a promising foundation. For successful translation to blood banks, we envision that these platforms must address three main technical challenges in terms of sampling, detection and detection miniaturization, and integration with blood units.

Firstly, the sampling of RBC units should be performed without any contamination and any changes to the quality of the unit, *i.e.*, in a non-invasive manner. Further, the sampling should be representative of the entire unit and bias free. Given that an advantage of LOCs is the use of low volumes compared to traditional assays, this implies the use of repeated or continuous sampling to ensure robust and representative measurements. While current sampling approaches such as sampling site couples or sterile connected containers can be used in early non-integrated versions of LOC safety assessments, they are cumbersome and impractical for the long-term integrated and streamlined

vision for RBC safety assessment. In this regard, engineering solutions providing small volume, and one-way / contamination-free sampling from inside the RBC units will be necessary.

Secondly, the detection of biomarkers for quality assessments should be performed in reasonably miniaturized platforms suitable for integration with 180-500 ml storage bags. Further, the ideal set of quality indices for RBC efficacy might necessitate multiple different detection modalities, which may pose additional challenges for achieving a reasonably small footprint for the integrated LOC platforms. Currently most of the research LOC platforms for RBC analyses feature cumbersome detection techniques such as benchtop microscopic imaging. Miniaturization and integration of detection on LOCs requires advanced engineering approaches. Reassuringly, many examples of commercially available LOCs that are stand-alone and portable demonstrate feasibility. Furthermore, continuous advances in microelectronics and micro-electro-mechanical systems (MEMS) devices and micro/nano fabrication are expected to enable manufacturing of miniaturized detection techniques. A common limitation for LOC platforms used in research for RBC assessment thus far has been their inability to work with the high hematocrit samples that are the norm for packed RBC units. This detection-related limitation can likely be overcome with on-chip manipulation, dilution, and multiplexing approaches.

Once the best strategy to perform aseptic and non-invasive sampling is established and the LOC platforms are sufficiently miniaturized, the next step will be integration of these platforms with the storage bags. We envision two routes for this integration. The first features a fully closed system where sampled volumes are routed to an LOC platform that is integrally manufactured with the blood bags. The second approach features a modular cartridge-based system that can accommodate different LOC platform designs through a universal fluid coupling port. The latter approach might allow rapid iteration of the platform technologies without affecting the design of the rest of the blood bag unit. The final engineering step is to integrate user-friendly reporting of the generated data through wireless technologies and cloud-based data management approaches.

Beyond the technical and practical challenges, the economics of integrating LOC platforms into the transfusion medicine workflow needs to be addressed. This challenge can be overcome by economies of scale and rapid advances in fabrication of microsystems technologies, which are expected to bring the unit costs down. The rapid cost and size reduction that we have witnessed during the microprocessor revolution is a prime example of how similar economic challenges have been overcome. User-friendly implementation of LOC platforms should impose few new skillset requirements, which will ease the adoption process.

Concluding Remarks

Lab-on-a-chip technologies can ensure a more successful transfusion workflow by enabling objective assessment of stored RBC units using quality metrics identified by -omics and machine learning, thus ushering in a new era of precision transfusion medicine. Addressing the potential and challenges of precision transfusion medicine will facilitate the proposed convergent future, as depicted in **Fig. 4**. This will require all stakeholders (including experts in blood banking, biopreservation, transfusion medicine, -omics, machine learning, bioengineering, ethics, regulation, and ultimately patients) to participate in multidisciplinary discussions and collaborations rather than their current isolated efforts. This Perspective – where we have a diverse team of such stakeholders – is a step towards this envisioned era of precision in transfusion medicine.

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Competing Interests

U.A.G. and Case Western Reserve University (CWRU) have financial interests in Hemex Health Inc. U. A. G., E. K., and CWRU have financial interests in BioChip Labs Inc. U.A.G. and CWRU have financial interests in Xatek Inc. U. A. G. has financial interests in DxNow Inc. The competing interests of CWRU employees are managed by the Conflict of Interests Committee according to a Conflict-of-Interest Management Plan. The study was conducted prior to E.K. employment at IDEXX Laboratories, and the ideas expressed are not in his capacity as an IDEXX employee. A. D. serves on the Scientific Advisory Board of Hemanext Inc and Macopharma Inc; his competing interests are managed by the University of Colorado in accordance with their conflict-of-interest policies. M.T. and S.N.T. serve on the Scientific Advisory Board of Sylvatica Biotech Inc. The competing interests of Massachusetts General Hospital employees are managed by the Mass General Brigham in accordance with their conflict-of-interest policies. The remaining authors declare no competing interests.

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Legends

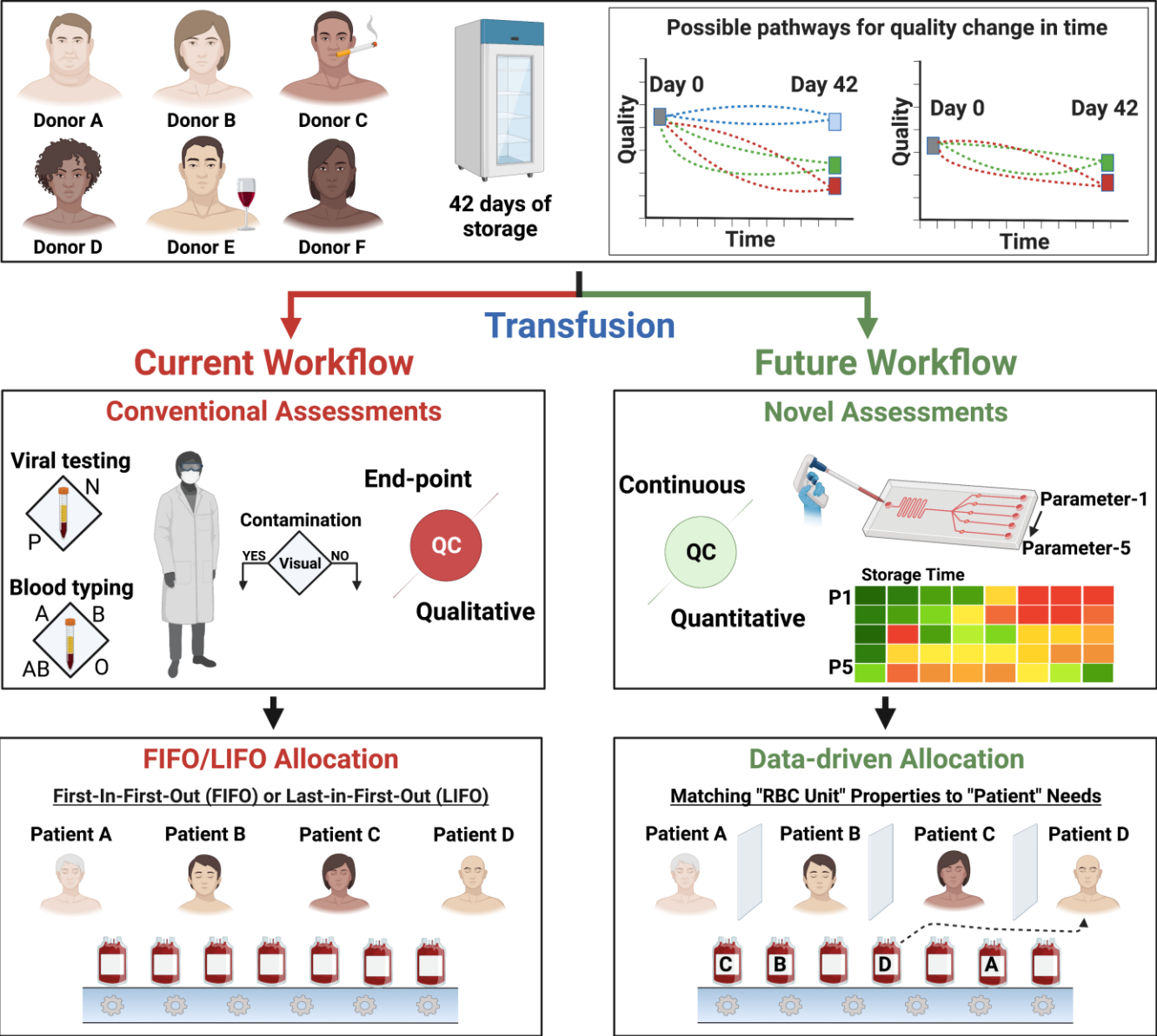
Figure 1. The current and proposed future workflow for RBC storage and transfusion medicine. Donors vary in sex, age, ethnicity, genetic disposition, body mass index, metabolism, and habits. These variations can lead to different initial quality levels for donated RBCs and to different quality changes during storage. Currently, blood banks store donated RBCs for 42 days at 4 °C. This storage can induce injuries collectively termed the “storage lesions” in a donor-, time-, and processing-dependent manner. Thus, the injury to each stored RBC unit can be unique. The current quality assessments performed on individual RBC units before transfusion are infectious disease marker testing and donor screening, blood typing, and visual inspection for bacterial contamination. These assessments are agreed upon and mandated by health agencies worldwide – including the FDA and CDC. RBC units are generally allocated following the “first-in-first-out” protocol. However, this workflow may result in post-transfusion complications and significantly reduced efficacy. The advances in lab-on-a-chip technologies armed with information from -omics and machine learning studies offer a better alternative. We envision a future where each RBC unit is assessed continuously during storage using multiple quality metrics based on objective unbiased measurements via lab-on-a-chip technologies. Patients with different medical conditions will then be administered the right RBC unit via data-driven allocation. Such an approach will maximize the efficacy of transfusion therapies and reduce complications.

Figure 2. Donor-, time-, and processing-dependent red blood cell (RBC) storage lesions. RBCs undergo various injuries during storage in their biochemical content, oxidative state, and morphology. Major biochemical changes include the depletion of ATP and 2-3 DPG. This is accompanied by an increase in reactive oxygen species (ROS) damaging proteins and lipids (through increased malondialdehyde – MDA), among other injuries affecting the cellular integrity. These injuries are often observed as irreversible RBC morphological changes resulting in loss of integrity (hemolysis), deformability, and discoid morphology. While these alterations have a general time-dependent trend, they also depend on the donor and the initial blood processing, which varies among blood collection centers. These donor-, time-, and processing-dependent changes are not monitored in today’s practice of blood banking.

Figure 3. Lab-on-a-chip (LOC) platforms for red blood cell (RBC) quality assessment. Figure (a) shows different aspects, components, and materials of lab-on-a-chip platforms from design and fabrication to operation (flow) and sensing. Such platforms enable detection of various biomarkers ranging from cells and proteins to ions. Figure (b) shows a schematic representation of one of the most commonly used RBC deformability measurements using LOC platforms. Such platforms use microfluidic constrictions to quantify the degree of deformability.

Figure 4. Convergent future of stored red blood cell assessment towards precision transfusion. Novel -omics and machine learning technologies have recently been used to assess RBC storage lesions. We envision that the integration of these technologies will identify key quality metrics (quality indices) for stored RBCs in the near future. These quality indices can then guide lab-on-a-chip platforms armed with novel biosensors to continuously monitor stored RBC quality and then match the properties of the RBC unit to the needs of the patient.

Donation and Storage



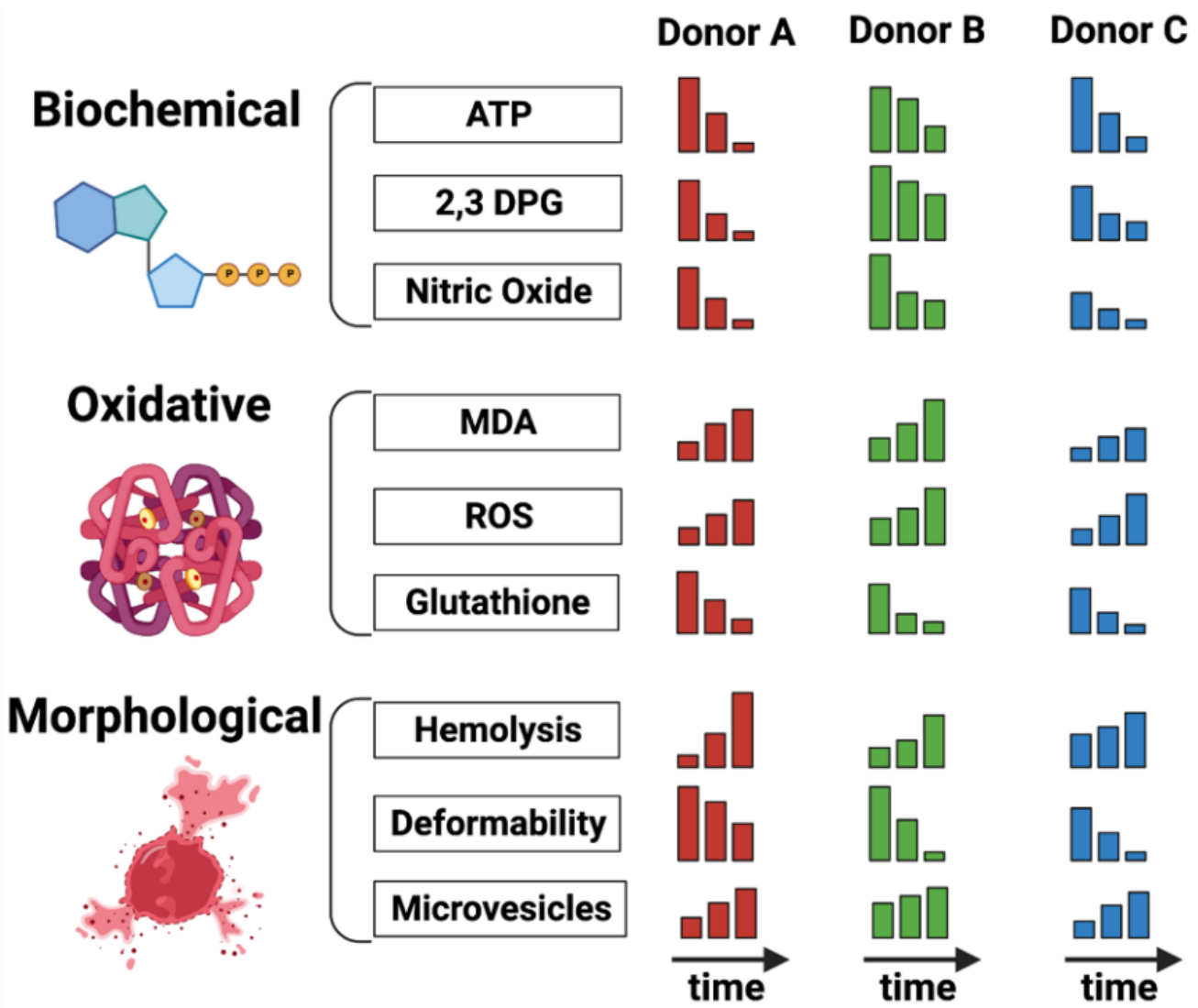


Figure 2

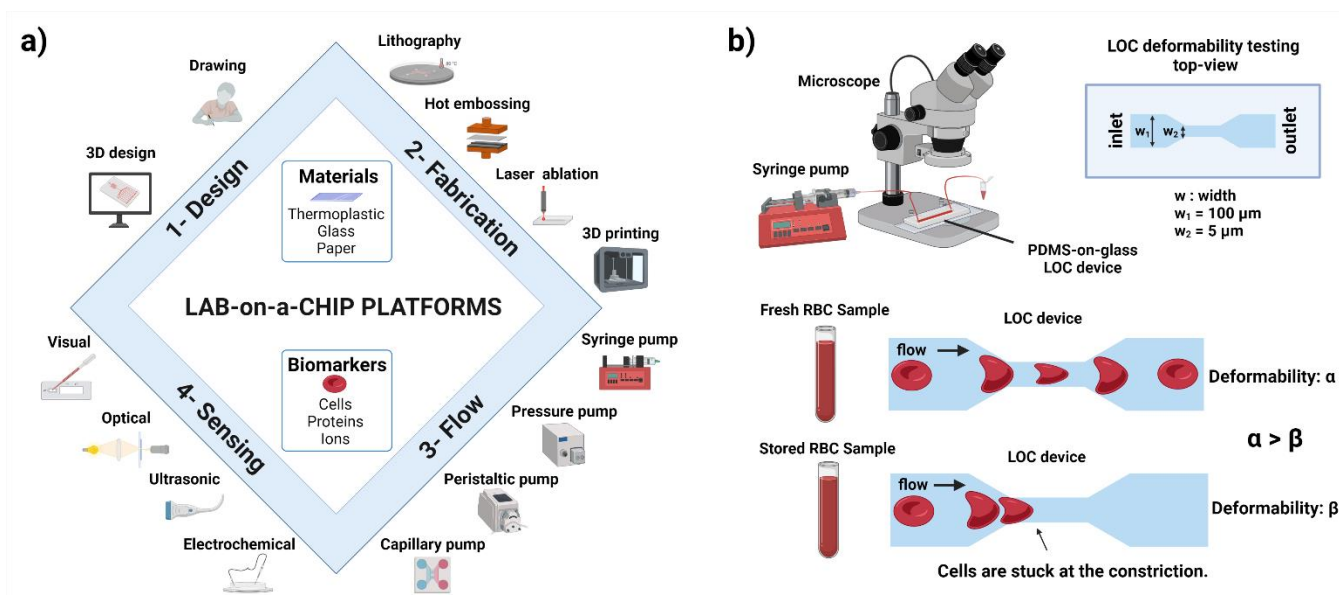


Figure 3

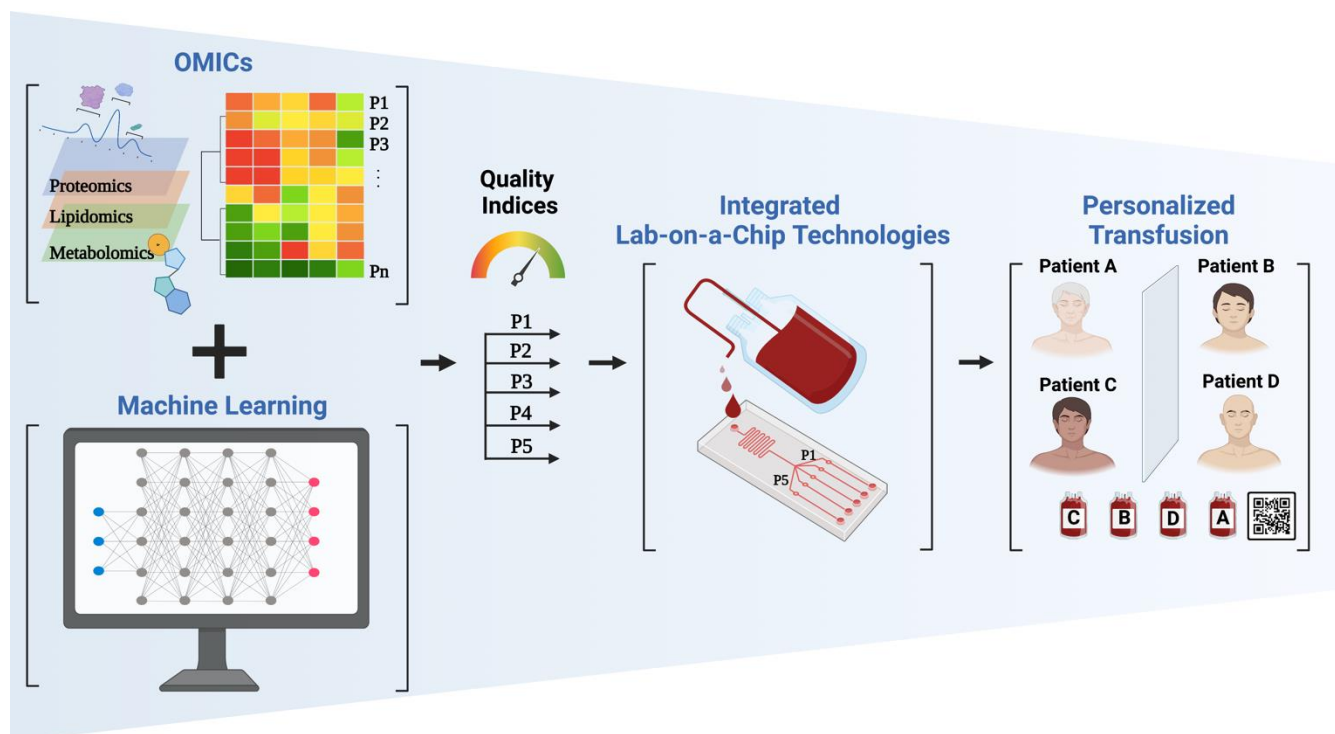


Figure 4