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Commentary

Third-Generation Sequencing in Clinical Practice: The New Era of Precision Medicine?

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Abstract: In the last decades, the spreading of next-generation sequencing (NGS) in clinical practice has considerably increased the genomic knowledge of several disorders. The recent advent of third-generation sequencing is transforming the standard way of conceiving clinical genomics, overcom-ing the main limits of conventional NGS technologies and achieving challenges so far considered unreasonable. What was impracticable only a few years ago, in terms of potential and affordability, now is becoming achievable. The new sequencing era will improve diagnostic and therapeutic ap-proaches, providing clinicians with valid support in their practice.

Keywords: third-generation sequencing; nanopore sequencing; clinical practice; infectious diseases; inherited disorders; cancer diseases

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Citation: Cumbo, C.; Minervini, C.F.; Albano, F. Third-Generation Sequencing in Clinical Practice: The New Era of Precision Medicine? *Appl. Sci.* **2022**, *12*, 6058. https://doi.org/10.3390/app12126058

Academic Editor: Hans Peter Deigner

Received: 26 April 2022
Accepted: 31 May 2022
Published: 15 June 2022

1. Introduction

Ten years ago, Oxford Nanopore Technologies (ONT) and Pacific Biosciences (PacBio) released their first long-read sequencers, paving the way for the "third-generation sequencing" (TGS) age [1]. The ability to produce long-reads is the distinctive advantage of both technologies, overcoming all known difficulties of conventional short-read sequencing and allowing to characterize complex genomic regions. ONT devices perform a nanopore-based sequencing (NS), on the contrary, PacBio platforms are based on a "single-molecule real-time" (SMRT) approach [1]. Later, Illumina and 10X Genomics developed two "synthetic long-reads" (SLRs) strategies, not considered TGS methods, because based on short-read technologies [1]. Nowadays, TGS protocols and pipelines are not yet standardized and suffer from a lower accuracy than conventional next-generation sequencing (NGS) strategies (i.e., Illumina and Ion Torrent). Despite this, in the last decade, several approaches have been performed in "-omic" sciences. Clinical genomics arises among them, with its main applications in infectious diseases, and inherited and oncologic disorders. Herein, we will discuss the main ONT challenges, a technology that offers great promise in the precision medicine era!

2. Infectious Diseases

Undoubtedly, the study of infectious diseases represents the main ONT application in clinical practice. Despite its short life, the technology has yet to be applied worldwide to support the rapid identification and control of Ebola, Zika, Yellow Fever, Swine flu, and COVID-19 disease outbreaks, making genomic epidemiology fast, easy, and cheap.

In April 2015, three ONT MinION platforms, four laptops, a thermocycler, a heat block, pipettes, and reagents were packed into less than 50 kg of standard airline travel luggage, transported to Guinea, and used for real-time genomic surveillance (less than 24 h from sample receiving) of the ongoing Ebola epidemy [2]. In June 2016, the ZiBRA project used MinION platforms to undertake fieldwork in a mobile laboratory travelling across five federal states in the Northeast region of Brazil, with the highest numbers of notified Zika cases [3].

But the real worldwide use of nanopore technology is still ongoing. From the initial characterization of the SARS-CoV-2 virus genome to rapid variants detection, NS has been widely used to combat the spread of COVID-19 disease [4]. ONT allows the fastest SARS-CoV-2 sequencing (<8 h from RNA extraction to answer) with the best scalability on three different platforms (MinION, GridION and PromethION): from 12 (MinION) to >1000 (PromethION) samples in a single run, with cost as low as $9.55 per sample [5]. Data produced on NS platforms become readily available to the research community on public databases such as GISAID [4,6] speeding up the diffusion of SARS-CoV-2 genotype and the worldwide knowledge of its variants.

Genomic epidemiology, powered by ONT rapidity and accessibility, provides vital information and time savings essential for rapid disease identification and control; furthermore, it supports precise tracking of pathogen evolution, allowing the potential association of novel variants with disease severity, transmission, and therapeutic efficacy. Last but not least, ONT platforms enable real-time metagenomic sequencing approaches, providing in COVID-19 patients in intensive care units the rapid identification of secondary infections, antimicrobial resistance, and nosocomial transmission [7].

ONT's success in infectious diseases management arises because of its affordability and the ability to generate long-reads. In fact, the capability to produce reads of >4 Mb greatly simplifies the assembly of microbial genomes, overcoming all known difficulties of short-reads (typically 150–300 bps) sequencing and allowing the sequencing of smaller genomes in single reads, negating the need for assembly [8]. A true revolution in the study and management of infectious diseases!

3. Inherited Disorders

Less widespread but still valid is the introduction of NS in human genetics, with numerous potential applications in diagnosing inherited disorders.

Between December 2020 and May 2021, at two hospitals in Stanford, California, 12 cases representative of persons living in the United States with respect to race, ethnic group, and sex were enrolled. Whole-genome NS was performed, and five of them obtained an initial genetic diagnosis in less than ten hours from the blood sample arrival [9]. Between them, it is emblematic the case of a 3-month-old full-term infant who presented in status epilepticus, for which a genetic diagnosis of a Poirier–Bienvenu neurodevelopmental syndrome (a *CSNK2B*-related disorder) was made in less than nine hours from enrollment (it takes more than two weeks by standard methods). The ultrarapid result obtained froze further planned diagnostic testing, simplified counseling, and prognostication, and aided in epilepsy management [9].

In addition to rapidity, the ability of NS to characterize tandem repeats (TR) is the other relevant advantage of ONT approaches in the diagnosis of genetic disorders. TR are implicated in numerous diseases, including Huntington's, frontotemporal dementia, and amyotrophic lateral sclerosis. Sometimes TR can cause disease through their length, sequence, or base modifications; these are all features often difficult, if not impossible, to be assessed with conventional methods. Long-reads NS allows spanning large TR, showing their size, sequence, and the presence of base modifications [10].

In the complex scenario of inherited disorders, it is mandatory to mention preimplantation genetic testing (PGT), for which both NS rapidity and detection ability are crucial. Standard molecular PGT (such as microarray and short-read sequencing) cannot distinguish embryos carrying balanced rearrangements from those with a normal karyotype, worsening the outcome of assisted reproduction. NS can detect these aberrations identifying exact breakpoints and allowing a haplotype linkage analysis [11]. Furthermore, at Columbia University Medical Center, it was shown the NS ability to perform in 20 min a complete aneuploidy screening and, in a few hours, large copy number variations (CNVs) or mosaic aneuploidy detection [12]. In contrast to existing assays, which take more than

12 h, ONT introduction in PGT could allow same-day testing and embryo transfer, thus avoiding the need for more complex and expensive techniques or embryo freezing [12]. A strategy that is cheaper, more accurate than conventional approaches, and that allows for same-day embryo transfer could be the future of PGT.

4. Cancer Diseases

The last clinical context where ONT performances have been tested in the last decade is oncologic disorders. In fact, the study of cancer genetics often concerns different genomic and epigenomic aberrations [i.e., single nucleotide variants (SNVs), insertions/deletions, CNVs, structural variants (SVs), methylation patterns] all detectable by NS.

Recently, it was demonstrated how NS combined with Cas9-based target enrichment enabled the analysis of ten clinically-relevant cancer-associated loci: *BRAF*, *KRAS*, and *TP53* SNVs, chromosomes 5 and 7 deletions, and the methylation status of *GPX1*, *GSTP1*, *KRT19*, *SLC12A4*, *TPM2* [13]. To date, no other single technology can address the same issues simultaneously.

Among oncologic disorders, NS has been widely tested in the study of blood cancers, malignancies often associated with genomic aberrations, for which the technology offers a wide range of chances [14,15]. Once again, long-read production and rapidity are advantages of ONT. The ability to produce long-reads simplifies the SVs characterization and, in particular, detects fusion genes (i.e., *BCR-ABL1*, *PML-RARA*, *AML1-ETO*, *CBFB-MYH11*) frequently observed in hematological malignancies [16–18]. On the other hand, NS could significantly speed up the diagnostic procedure of acute leukemias, whose timeliness is essential and could impact the patient outcome. The possibility to study (in 24 h) the most frequently mutated genes (*NPM1*, *FLT3*, *CEBPA*, *TP53*, *IDH1*, and *IDH2*) in adult acute myeloid leukemia (AML) is an example of the ONT capability to upgrade the AML diagnostics, improving the patient's management [19]. In the near future, simple, cheap, and fast sequencing strategies could deepen the knowledge of cancer genetics, increasing the power of precision medicine and the patients' chances of survival.

5. Conclusions

Although still far from its coming into clinical practice, ONT approaches have widely shown their numerous advantages in the last decade, but all that glitters is not gold! The main ONT downside remains the accuracy. The last chemistry update (Q20+) has improved to a median value of 99% (not yet comparable to conventional sequencing technologies); moreover, NS data analysis and interpretation are not ever user-friendly, and bioinformatics expertise is often necessary for the post-sequencing analytical step. All these limitations could hinder the diffusion of the technology in its different areas of application, not fully exploiting its potential. In fact, in spite of conventional NGS approaches, widely tested and adopted in research and clinics, TGS is still away from its introduction in diagnostic procedures. Despite this, it is transforming the standard way of conceiving clinical genomics, achieving challenges so far considered unreasonable. The constant improvements in the technology and the development of easy-to-use analytical interfaces could pave the way for a new sequencing era, allowing the analysis of "anything, by anyone, anywhere"!

Author Contributions: Conceptualization, C.C. and F.A.; writing—review and editing, C.C. and C.F.M.; supervision, F.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: This work was supported by "Associazione Italiana contro le Leucemie (AIL)-BARI".

Conflicts of Interest: The authors declare no conflict of interest.

References

1. van Dijk, E.L.; Jaszczyszyn, Y.; Naquin, D.; Thermes, C. The Third Revolution in Sequencing Technology. *Trends Genet.* **2018**, *34*, 666–681. [CrossRef] [PubMed]
2. Quick, J.; Loman, N.J.; Duraffour, S.; Simpson, J.T.; Severi, E.; Cowley, L.; Bore, J.A.; Koundouno, R.; Dudas, G.; Mikhail, A.; et al. Real-time, portable genome sequencing for Ebola surveillance. *Nature* **2016**, *530*, 228–232. [CrossRef] [PubMed]
3. Faria, N.R.; Sabino, E.C.; Nunes, M.R.T.; Alcantara, L.C.J.; Loman, N.J.; Pybus, O.G. Mobile real-time surveillance of Zika virus in Brazil. *Genome Med.* **2016**, *8*, 2–5. [CrossRef] [PubMed]
4. Zhu, N.; Zhang, D.; Wang, W.; Li, X.; Yang, B.; Song, J.; Zhao, X.; Huang, B.; Shi, W.; Lu, R.; et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N. Engl. J. Med.* **2020**, *382*, 727–733. [CrossRef] [PubMed]
5. COVID-19. Available online: https://nanoporetech.com/covid-19 (accessed on 25 April 2022).
6. GISAID—Initiative. Available online: https://www.gisaid.org/ (accessed on 25 April 2022).
7. Charalampous, T.; Alcolea-Medina, A.; Snell, L.B.; Williams, T.G.; Batra, R.; Camporota, L.; Meadows, C.I.S.; Wyncoll, D.; Barrett, N.A.; Hemsley, C.J.; et al. Application of respiratory metagenomics for COVID-19 patients on the intensive care unit to inform appropriate initial antimicrobial treatment and rapid detection of nosocomial transmission. *medRxiv* **2022**. [CrossRef]
8. Genome Assembly | Oxford Nanopore Technologies. Available online: https://nanoporetech.com/applications/investigation/assembly (accessed on 25 April 2022).
9. Gorzynski, J.E.; Goenka, S.D.; Shafin, K.; Jensen, T.D.; Fisk, D.G.; Grove, M.E.; Spiteri, E.; Pesout, T.; Monlong, J.; Baid, G.; et al. Ultrarapid Nanopore Genome Sequencing in a Critical Care Setting. *N. Engl. J. Med.* **2022**, *386*, 700–702. [CrossRef] [PubMed]
10. De Roeck, A.; De Coster, W.; Bossaerts, L.; Cacace, R.; De Pooter, T.; Van Dongen, J.; D'Hert, S.; De Rijk, P.; Strazisar, M.; Van Broeckhoven, C.; et al. NanoSatellite: Accurate characterization of expanded tandem repeat length and sequence through whole genome long-read sequencing on PromethION. *Genome Biol.* **2019**, *20*, 239. [CrossRef] [PubMed]
11. Zhang, S.; Liang, F.; Lei, C.; Wu, J.; Fu, J.; Yang, Q.; Luo, X.; Yu, G.; Wang, D.; Zhang, Y.; et al. Long-read sequencing and haplotype linkage analysis enabled preimplantation genetic testing for patients carrying pathogenic inversions. *J. Med. Genet.* **2019**, *56*, 741–749. [CrossRef] [PubMed]
12. Wei, S.; Weiss, Z.R.; Gaur, P.; Forman, E.; Williams, Z. Rapid preimplantation genetic screening using a handheld, nanopore-based DNA sequencer. *Fertil. Steril.* **2018**, *110*, 910–916.e2. [CrossRef] [PubMed]
13. Gilpatrick, T.; Lee, I.; Graham, J.E.; Raimondeau, E.; Bowen, R.; Heron, A.; Downs, B.; Sukumar, S.; Sedlazeck, F.J.; Timp, W. Targeted nanopore sequencing with Cas9-guided adapter ligation. *Nat. Biotechnol.* **2020**, *38*, 433–438. [CrossRef]
14. Minervini, C.F.; Cumbo, C.; Orsini, P.; Anelli, L.; Zagaria, A.; Specchia, G.; Albano, F. Nanopore Sequencing in Blood Diseases: A Wide Range of Opportunities. *Front. Genet.* **2020**, *11*, 76. [CrossRef] [PubMed]
15. Orsini, P.; Minervini, C.F.; Cumbo, C.; Anelli, L.; Zagaria, A.; Minervini, A.; Coccaro, N.; Tota, G.; Casieri, P.; Impera, L.; et al. Design and MinION testing of a nanopore targeted gene sequencing panel for chronic lymphocytic leukemia. *Sci. Rep.* **2018**, *8*, 11798. [CrossRef] [PubMed]
16. Jeck, W.R.; Lee, J.; Robinson, H.; Le, L.P.; Iafrate, A.J.; Nardi, V. A Nanopore Sequencing–Based Assay for Rapid Detection of Gene Fusions. *J. Mol. Diagn.* **2019**, *21*, 58–69. [CrossRef] [PubMed]
17. Minervini, C.F.; Cumbo, C.; Orsini, P.; Anelli, L.; Zagaria, A.; Impera, L.; Coccaro, N.; Brunetti, C.; Minervini, A.; Casieri, P.; et al. Mutational analysis in BCR-ABL1 positive leukemia by deep sequencing based on nanopore MinION technology. *Exp. Mol. Pathol.* **2017**, *103*, 33–37. [CrossRef] [PubMed]
18. Cumbo, C.; Impera, L.; Minervini, C.F.; Orsini, P.; Anelli, L.; Zagaria, A.; Coccaro, N.; Tota, G.; Minervini, A.; Casieri, P.; et al. Genomic BCR-ABL1 breakpoint characterization by a multistrategy approach for "personalized monitoring" of residual disease in chronic myeloid leukemia patients. *Oncotarget* **2018**, *9*, 10978. [CrossRef] [PubMed]
19. Cumbo, C.; Minervini, C.F.; Orsini, P.; Anelli, L.; Zagaria, A.; Minervini, A.; Coccaro, N.; Impera, L.; Tota, G.; Parciante, E.; et al. Nanopore Targeted Sequencing for Rapid Gene Mutations Detection in Acute Myeloid Leukemia. *Genes* **2019**, *10*, 1026. [CrossRef] [PubMed]